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THE BRITISH JOURNAL
OF
EXPERIMENTAL PATHOLOGY

ON THE EFFECTS OF CHANGES IN INTRAVENTRICULAR PRESSURE AND FILLING ON THE VENTRICULAR RHYTHM IN PARTIAL AND COMPLETE HEART BLOCK.

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AND
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Received for publication November 22nd, 1921.

RECENTLY one of us has had the opportunity of observing a number of clinical cases suffering from auriculo-ventricular block, and our attention was drawn in particular to the change in ventricular frequency which occurred with or without the onset of syncope.

With the aid of modern instruments the mechanism of any given change of rhythm may be determined in cases of both partial and complete auriculo-ventricular block; on the other hand, the actual causes of the alterations in rhythm are nearly always obscure.

In two cases suffering from complete auriculo-ventricular block (confirmed histologically), the idio-ventricular rhythm was interrupted by groups of rapid ventricular systoles, periods of asystole intervening between each group. In one case the ventricular frequency would sometimes be as high as 130 per minute at the commencement of the group and gradually slow down to about 70 per minute before stopping altogether. The ventricular pauses lasted from a few seconds to three minutes. The ventricular beats were counted with the stethoscope over the præcordium, and we presume the rhythm was due to ectopic beats arising in the ventricle in the same manner as reported by Cohn and Lewis (1912–13).

The effect upon idio-ventricular rhythm of rapid artificial stimuli applied to the ventricle has been studied experimentally by Erlanger and Hirschfelder (1905–6). They found that cessation of a new and more rapid artificial rhythm was followed by a ventricular pause, the idio-ventricular rhythm taking time to develop again. In the report of Cohn and Lewis the idio-ventricular rhythm was interrupted by a number of premature beats arising in the ventricle; at the end of the ectopic rhythm there was a ventricular pause

before the idio-ventricular rhythm developed; they pointed out the resemblance between their case and the condition studied experimentally by Erlanger and Hirschfelder. Cushny (1911–12), in his researches on the mammalian heart of the cat in which the main stem of the auriculo-ventricular bundle had been cut, confirmed the findings of Erlanger and Hirschfelder, and in addition made important observations upon the conditions which govern the length of the ventricular pause following the stoppage of artificial stimuli applied to the ventricles. He stated that the length of the ventricular pause depended upon the duration and rapidity of stimulation preceding the pause; increase in either the duration or rate of artificial stimuli prolonged the ventricular pause. The excitability of the ventricle to electric stimuli remained unchanged during the ventricular standstill, and he concluded that the processes concerned with impulse formation and with excitability were different.

In cases of complete auriculo-ventricular block it is only rarely that one can discover a predisposing cause for the changes in ventricular frequency. The effect of nervous influences is difficult to eliminate, but cases have been reported where the ventricular rhythm appeared to have been altered by them. In the majority of cases, and in those coming under our observation, no determining causes of the changes in ventricular rate, except those due to exercise and excitement, could be discovered. Investigations upon the effect of mechanical conditions of the circulation upon the ventricular rhythm in animals suffering from auriculo-ventricular block have not yet been carried out; this paper deals with experiments bearing upon this problem.

The heart-lung preparation described by Knowlton and Starling (1912) has been used throughout the research; with this preparation both the systolic and diastolic volumes of the heart may be varied at will by altering the venous inflow and arterial resistance. The intra-ventricular pressure is also changed by any alteration in the arterial resistance. Reflex nervous influences are absent—an essential control in the experiments. Hering (1911) has questioned that electrical excitability can in all cases be regarded as a measure of the natural excitability of the heart; therefore if any changes in ventricular rhythm occurred as a result of altering the mechanical conditions of the circulation, there would be no doubt that they were due to the excitability of the heart under the existing conditions of the experiment.

DESCRIPTIONS OF EXPERIMENTS.

In all, eight experiments were performed; the animals were anæsthetised with chloroform and ether after a preliminary injection of morphia; a 2 per cent. solution of chloralose in ·9 per cent. NaCl was then given intravenously, the dose being one decigramme per kilogramme body-weight.

In all the experiments, with the exception of No. 1, which was carried out in the usual way, a slight modification of technique enabled us to dispense with hirudin, the blood-clotting being prevented by the use of defibrinated blood.

The actual method is as follows: The heart-lung preparation is carried out as usual until everything is ready for the circulation to be switched over through the artificial resistance. The venous reservoir is filled with defibrinated blood from another dog previously bled for the purpose. Just before the aorta is ligatured, the blood in the heart is allowed to flow out through a side tube attached to the cannula which is inserted in the innominate artery. The

aorta and inferior vena cava are then quickly tied, and defibrinated blood is allowed to flow into the right auricle from the venous reservoir. The side tube in the innominate artery is kept open until the defibrinated blood from the venous reservoir has washed out all the animal's own blood from the heart and lungs; it is then closed, and simultaneously the tube from the innominate cannula to the artificial circulation is unclamped. The whole operation from the time the side tube in the innominate cannula is opened to the time when the artificial circulation is connected up takes about 15 seconds. If the heart is kept too long without a resistance to pump against, it will fail. As an additional safeguard to prevent the formation of clots, the blood may be collected for a few seconds from the side tube distal to the arterial resistance before it enters the venous reservoir. The animal's own blood is defibrinated by whipping, and added to the reservoir later. In one experiment carried out in this way the heart was found to be beating well after five hours.

In the heart block operation clamping of the auriculo-ventricular bundle was performed by the method described by Erlanger and Blackman (1909–10), a modified form of the heart clamp designed by Erlanger (1905) being used. The clamp was inserted into the right auricular appendix and the bundle crushed; the heart was then watched in order to determine whether auriculo-ventricular block had been produced; as a rule two or three attempts were necessary before the operation was successful.

Methods of recording.—At first attempts were made to record the mechanical contractions of the auricle and ventricle by connecting the right auricular appendix to a light writing lever and attaching a piston myocardiograph to the ventricle; cardiometer and spring tambour tracings were also taken from the ventricle. The blood-pressure was recorded by means of a Hürthle or mercury manometer. The mechanical disturbance of the ventricles consequent upon the variations in the conditions of the artificial circulation was so great as to render a sure interpretation of the ventricular rhythm uncertain. We therefore turned our attention to recording the heart-beats electro-cardiographically. An Einthoven string galvanometer of the standard pattern made by the Cambridge and Paul Scientific Instrument Company was used. The fibre resistance was 3000 ohms, deflection time less than 0·2 sec., and deflections generally 10 mm. per millivolt. Zinc electrodes bound with bandages impregnated with kaolin and ·9 per cent. saline were placed at the root of the neck and the under surface of the centre of the diaphragm. After the heart-lung preparation and the operation for auriculo-ventricular heart block had been completed the chest was closed. Unfortunately some of the curves are marred by the collapse and expansion of the lungs, which altered the position of the heart and the amount of surface in contact with the

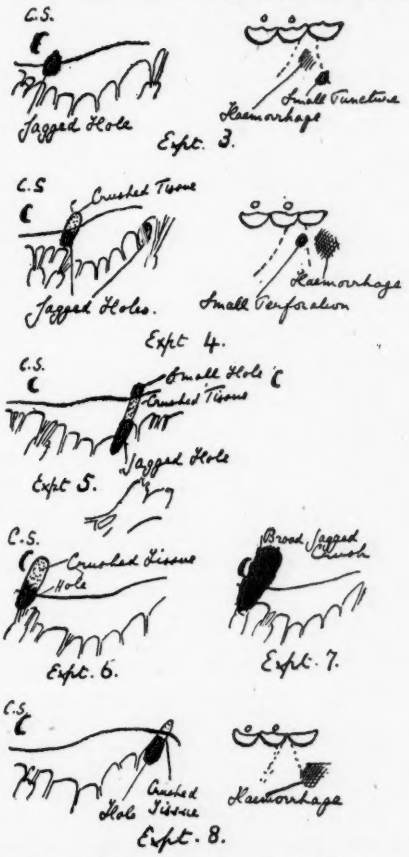

FIG. 7.—Sketches of lesion. About half natural size. C.S. = Coronary sinus.

adjacent tissues; this was more evident with an open pericardium. In later experiments the respirations were stopped during the fall of the photographic plate.

No attempt was made to examine the hearts histologically, as investigations upon the extent of the lesion and resultant effects upon the heart rhythm have been carried out by many workers. Sketches of the lesion were made and are seen in Fig. 7 (p. 3).

RESULTS.

Partial auriculo-ventricular heart block.—In two experiments (Nos. 5 and 8) partial heart block resulted. In Experiment 5 the block was complete at the beginning of the experiment, but gradually conduction of the auriculo-ventricular bundle improved until 1 : 1 rhythm with a prolonged P–R interval supervened. Both a large rise in arterial resistance (one reading) and a large increase in venous inflow (two) impaired the conduction of the auriculo-ventricular bundle (Plate I, fig. 1). The actual production of 2:1 auriculo-ventricular block (*b* in Plate I, fig. 1) appears to be due to the fact that every other auricular beat is premature, and the ventricle only responds to the normal rhythm. In this curve we have an example of auricular ectopic beats caused by increased work thrown upon the auricles. In Experiment 8, with the exception of the first record obtained, the rhythm varied between complete and 2 : 1 heart block; 2 : 1 auriculo-ventricular block always occurred with the systolic blood-pressure below 130 mm. Hg. (four), and complete heart block with the systolic blood-pressure above 130 mm. Hg. (six). Plate I, fig. 2, shows the effect of raising the mean blood-pressure from 125 to 185 mm. Hg. by changing the arterial resistance. With the higher blood-pressure the auriculo-ventricular block is complete, and the idio-ventricular rhythm is more frequent than the ventricular rate when 2 : 1 auriculo-ventricular block is present; there appears to be no doubt that the effect is not due to a change in temperature, as the auricular frequency is a little slower in the rhythm with complete auriculo-ventricular block. The effect of intra-ventricular pressure in relation to ventricular frequency will be discussed later.

Complete auriculo-ventricular block.—In Experiments 1, 2, 3, 4, 6 and 7, complete auriculo-ventricular block was produced. Mechanical records of the auricle and ventricle were taken in Experiments 1, 2 and 3; the nature of the irregularity, occurring under varying mechanical conditions of the circulation, could not be definitely ascertained and could only be inferred from the arterial pressure curve.

In Experiment 1, a rise in arterial resistance caused ventricular irregularity, as also did a rise in venous inflow; spontaneous disappearance of the new rhythm took place once. A fall in arterial resistance which was previously high caused disappearance of the ventricular irregularity (one).

In Experiment 2, during the first eight alterations of the artificial circulation ventricular irregularities were brought about by a rise (two) and a fall (one) in arterial resistance. A fall in arterial resistance initially high caused the irregularity to disappear (one), and no change in the ventricular rhythm occurred as a result of altering mechanical conditions four times. The succeeding ten alterations comprised a rise in arterial resistance (three), a fall in arterial resistance (five), a rise in venous inflow (one), and a fall in inflow (one); none of these changes were productive of irregularities of the

ventricle with the exception of a single ventricular premature beat, which occurred twice.

In Experiment 3, a rise in arterial resistance produced a ventricular irregularity, which disappeared on the resistance being lowered. An increase in venous inflow also caused ventricular irregularity, but it disappeared spontaneously (Plate I, fig. 3).

Electrocardiograms were taken in Experiments 4, 6 and 7.

In Experiment 4, Plate II, fig. 4, *a*, *b* and *c* show the effect of raising the inflow with a constant arterial resistance; an irregularity occurred, but only the last two beats have been caught. The irregularity disappeared without the venous inflow being reduced. The abnormal complexes in *b* in Plate I, fig. 2, may be due to premature beats of the ventricle arising in the right basal position of the heart, but the curves *a*, *b* and *c* in Plate II, fig. 4, are suggestive of incomplete bundle branch block as described by Wilson and Herrmann (1921). To determine the exact nature of the ventricular irregularity would require more information than we are able to produce, as in these experiments we were only concerned with the possibility of a change of rhythm. It is to be remembered that the leading off electrodes were from the root of the neck and diaphragm, the pericardium was open, so allowing of greater altération in the heart's axis, and also we have no records of the three standard leads. Our curves, therefore, give no certain indication of the path of the stimulus. In this experiment a rise in arterial resistance to 170 mm. Hg. did not bring about any change in the ventricular rhythm, but when the pericardium was opened a rise in arterial resistance to the same height caused an irregular and more frequent ventricular action (Plate II, fig. 4, *c*). The new rhythm disappeared spontaneously, an infrequent ventricular rhythm supervening. In this experiment we have an analogy to the cases previously described where the idio-ventricular rate is interrupted by a number of ventricular ectopic beats, cessation of the new rhythm being followed by an infrequent ventricular rate. The electrocardiogram in Plate II, fig. 4, *e*, is marred by respiratory effects.

In Experiment 6, an increase in the arterial resistance from 60 to 195 mm. Hg. produced a number of ventricular premature beats apparently arising in the left ventricle. In Plate III, fig. 5, *a*, *b*, shows the effect of an increase in venous inflow with a constant high arterial resistance. Plate III, fig. 5, *c*, *d*, shows the effect of reducing the arterial resistance and venous inflow when they are both initially high. It is possible that complete auriculo-ventricular heart block has given place to 2 : 1 auriculo-ventricular block in Plate III, fig. 5, *d*, but as the curves were not extended the rhythm is uncertain. The inter-ventricular cycles and the P–R intervals are marked on the curves. The fifth and sixth complexes in Plate III, fig. 5, *b*, at first sight suggest auriculo-ventricular nodal beats, but as there was reason to believe that extrinsic deflections were involved in this experiment, no certain interpretation of the complexes is possible.

In Experiment 7, a more frequent ectopic ventricular rhythm occurred as the result of lowering an initially high arterial resistance. The new rhythm is due to premature ventricular beats probably arising in the left ventricle. A further decrease of arterial resistance to zero, which gave a mean blood-pressure of approximately 40 mm. Hg., caused the premature beats to dis-

appear and the supraventricular rhythm returned, but at a diminished frequency when compared with the initial rate (Plate III, fig. 6, *a*).

DISCUSSION OF RESULTS.

In attempting to arrive at an explanation of the attacks of temporary acceleration of ventricular rhythm occurring in subjects with complete heart-block, it must be remembered that division of the auriculo-ventricular bundle does not cut off the sympathetic nerve supply to the ventricles. This has been shown by Cullis (1913) for the cat, and in one experiment we found that after complete division of the bundle in the dog, we could produce acceleration of the idio-ventricular rhythm by stimulating the posterior branch of the ansa vieussenii. In this case we obtained an acceleration of ventricular rhythm from 48 to 60 per minute. It must therefore be presumed that this path is open also in patients with heart block, and there can be little doubt that many, if not most, of the cases of temporary acceleration of rhythm incident on exercise and possibly on emotional states may be ascribed to nervous impulses reaching the ventricles along sympathetic channels.

Lutembacher (1921) has shown that adrenalin accelerates the idio-ventricular rate in patients suffering from complete auriculo-ventricular block, but this acceleration is followed by a subsequent slowing of the pulse, so that the tendency to syncope is increased, and the administration of this drug is therefore dangerous in such cases. Adrenalin acts by a direct excitation of the sympathetic nerve-endings or the neuro-muscular junctions, but the course of events observed after its absorption is very similar to that we have described as occurring in man at the beginning of this paper.

There is, however, a further possible explanation which must be considered before ascribing all such alterations of rhythm to sympathetic influence. Ludwig and Luchsinger (1881), working on the isolated frog's ventricle, and Biedermann (1884) on the snail's heart, found that a rise in intra-ventricular pressure increased the frequency of beats, and Biedermann states that in the snail's heart this increased frequency was attended with a decrease in the systolic volume of the heart. In the mammalian heart Hering (1900) has shown that a sudden rise of ventricular pressure may give rise to ventricular premature beats. This could only be explained by ascribing a direct excitatory effect to the increased tension on the ventricular muscle, or, at any rate in the frog, to that part of the ventricle which was initiating the spontaneous beat. We know that in the isolated mammalian heart a rise of intra-ventricular tension, or indeed any other mechanical alteration in the conditions of the circulation, causes no change in cardiac rhythm, the sole means of altering the cardiac rhythm being by changing the temperature of the pace-maker. It seems possible that although the pace-maker in the sino-auricular node is insusceptible to changes in intra-auricular tension, this may be due to some structural arrangements preventing its constituent fibres being stretched by a rise of intra-auricular tension, or by increase of inflow into the auricles. When, after division of the auriculo-ventricular bundle, the ventricle is beating with its own rhythm, initiated either in the auriculo-ventricular node in the bundle itself or in other parts of the ventricular muscle, we might find a direct reaction of such vicarious pace-

makers to increased intra-ventricular tension or to increased volume, or stretching of the ventricles similar to that described by Ludwig and Luchsinger in the frog's ventricle. At any rate, the possibility had to be tested experimentally, since any such reaction of the ventricular muscle might be a contributory cause in the acceleration of ventricular rate observed in cases of complete heart-block as a result of exercise, *i. e.* under conditions where there will be an increased intra-ventricular pressure due to the rise of arterial pressure and possibly an increased ventricular volume.

In our experiments all intervention of nervous impulses was excluded by the conditions of the preparation itself, but in a considerable proportion of them we obtained a quickening of ventricular rhythm and extra beats, as a result of mechanical changes in the circulation. As a rule these could be evoked either by a considerable rise in the arterial resistance or by increasing the venous inflow into the heart. Both these conditions would cause a rise in the intra-ventricular pressure, as well as dilatation and stretching of ventricular muscle. But the fact that they were more easily evoked after the pericardium was freely open seems to indicate that the latter factor, *i.e.* the stretching of the ventricular muscle, was the more important of the two. Normally the pericardium limits the dilatation of the heart, and opening the pericardium, while rendering the heart more efficient for a time, brings this about by allowing increased dilatation and therefore increased length of cardiac muscle-fibre. Our experiments do not, however, permit us to accept this conclusion without reserve. They were all carried out within a short time of the division of the auriculo-ventricular bundle, *i.e.* there was a recent injury of this bundle and of the tissues in its vicinity, which might act as a source of irritation, and which might be susceptible of further tearing and damage when the pressure or filling was suddenly raised and the walls of the heart considerably dilated.

Lewis (1920) says: "It is possible that transient intensification of an inflammatory process in the bundle may sometimes provoke the attacks; an increase of pressure in the blood-vessels or lymph spaces of this region, by altering the tissue tensions, might abolish conduction in a bundle in which it was already precarious." We have shown that this is actually what happened in one of our cases of incomplete heart block, where rise of arterial pressure changed a 2 : 1 rhythm into complete block, but at the same time seemed to act as a direct excitation of the lesion, since the idio-ventricular rhythm was somewhat greater (78 per minute) than the previous 2 : 1 rhythm (60 per minute). The fact that in the experiments here recorded the accelerating influence on the ventricles of increased pressure or filling was most marked at the beginning of the experiments, and indeed often passed off while the pressure was still high, seems to indicate that the essential feature was not the increased pressure or stretching of the sound ventricular muscle, but the irritation due to further tearing or injury at the site of the recent lesion. We are thus probably dealing with a condition resembling some active progressive inflammatory lesion in the bundle. We regard this as the most probable explanation of our results, but we cannot exclude the possibility of a direct excitation of sound ventricular tissue until the experiments have been repeated, leaving some weeks between the division of the bundle and the investigation of effects of rise of pressure. We hope to carry out such experiments later.

CONCLUSIONS.

(1) In confirmation of other observers we have found that after complete division of the auriculo-ventricular bundle, acceleration of the idio-ventricular rhythm can be evoked by stimulation of the cardio-sympathetic nerves.

(2) In the heart separated altogether from nervous impulses, as in the heart-lung preparation, a rise of arterial pressure, or increased inflow into the heart, frequently causes increase in the idio-ventricular rhythm after complete division of the auriculo-ventricular bundle.

(3) It cannot yet be decided whether this result is due to the stimulating effects of increased tension or increased stretching of the ventricular wall, or to a direct irritant effect of the stretching on the recent lesion in the neighbourhood of the auriculo-ventricular bundle. Reasons are given for believing that the latter is the more likely explanation.

(4) After partial division of the auriculo-ventricular bundle, a rise of arterial pressure or increased filling may cause the block to become complete, the block becoming once more incomplete when the pressure or filling is reduced to its previous value.

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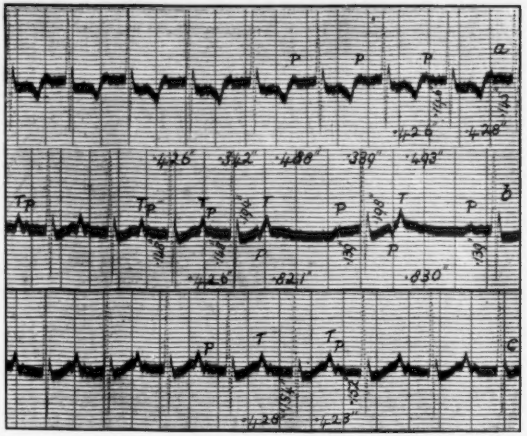

FIG. 1.—Exp. 5.

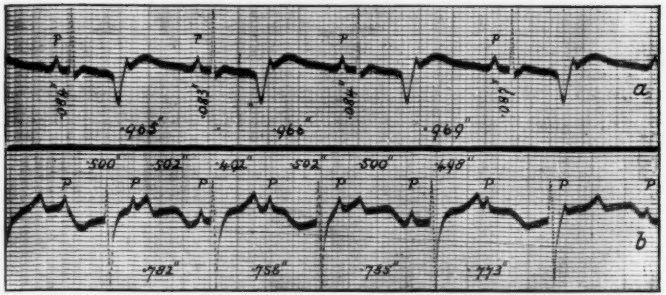

FIG. 2.—Exp. 8.

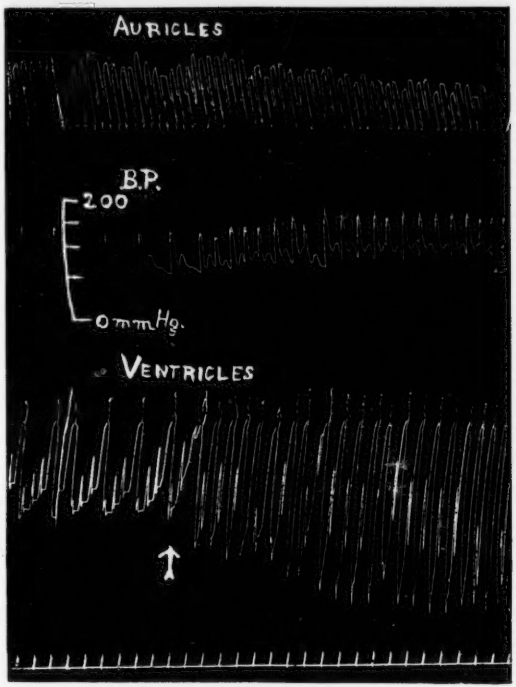

FIG. 3.—Exp. 3.

Plate I.

Daly and Starling. Partial and Complete Heart Block.

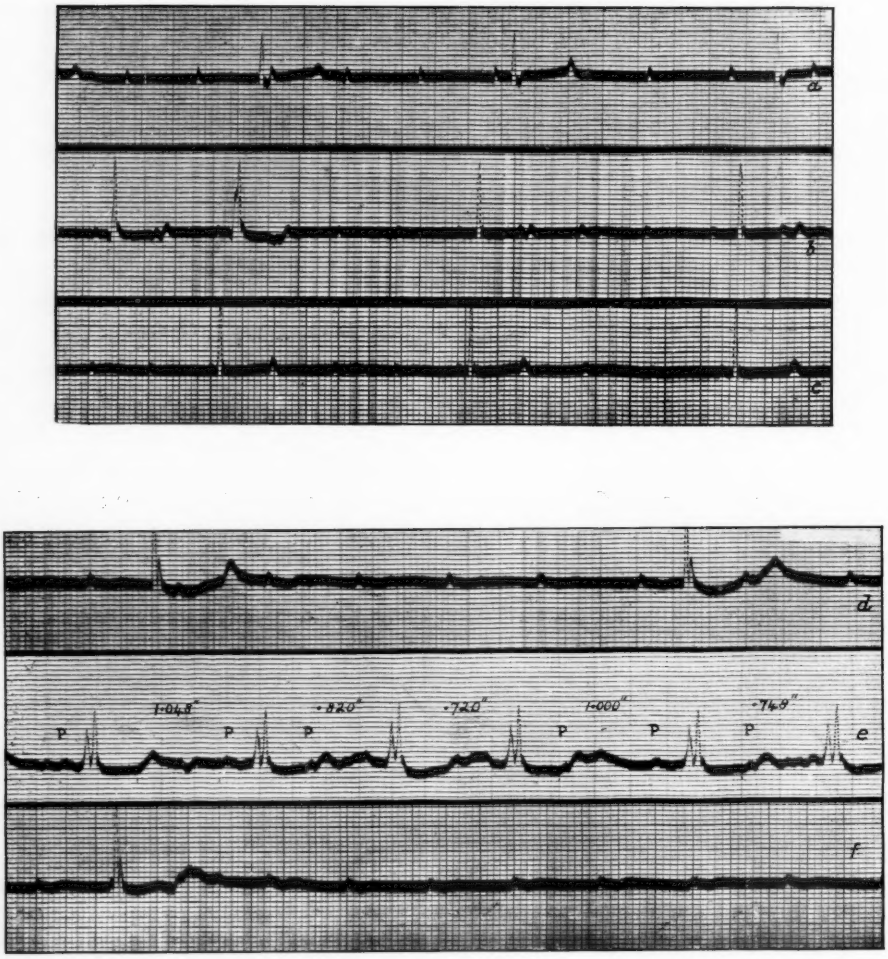

FIG. 4.—Exp. 4.

Plate II. Daly and Starling. Partial and Complete Heart Block.

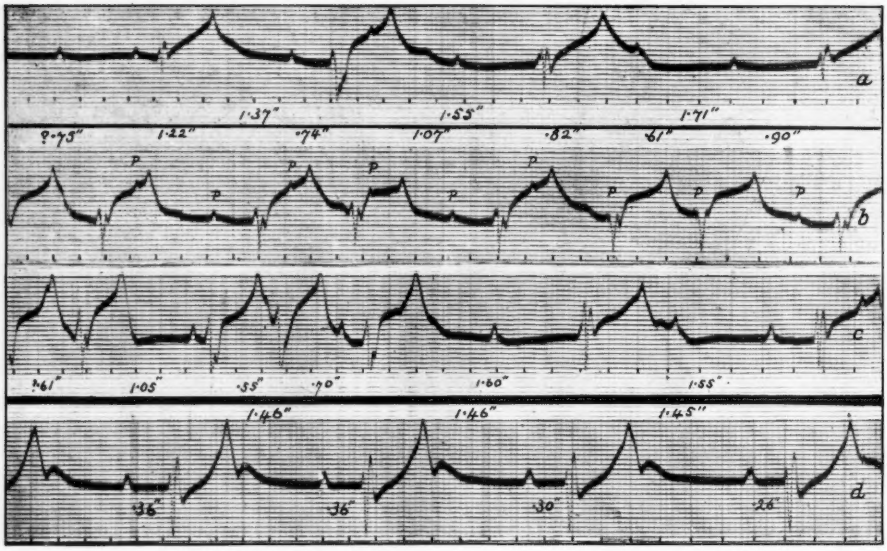

FIG. 5.—Exp. 6.

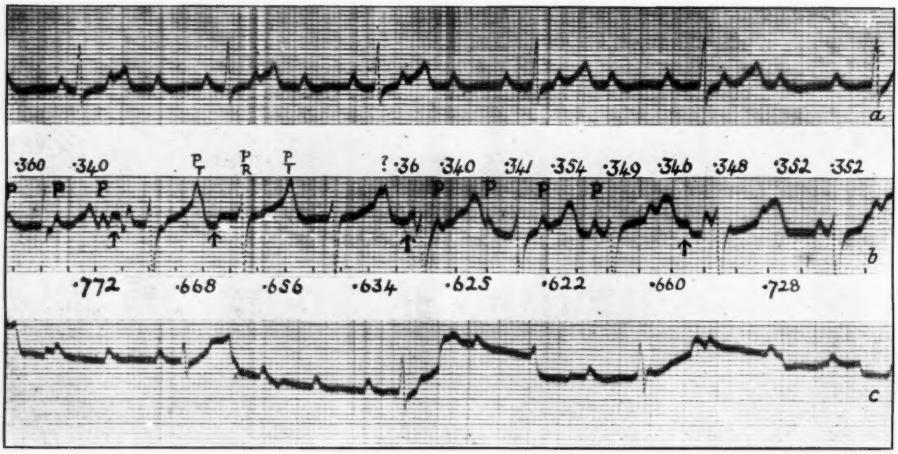

FIG. 6.—Exp. 7.

Plate III. Daly and Starling. Partial and Complete Heart Block.

EXPLANATION OF PLATES I–III, FIGS. 1–6.

PLATE I, FIG. 1.—Exp. 5. 6.5.'21. Dog, 7·0 kilo. Chloralose. Partial auriculo-ventricular block. Pericardium intact. Effect of raising the arterial resistance:

	O.P.	A.R.	Auricular rate per min.	Ventricular rate per min.	P-R interval.	Temp.
(*a*)	300	10	140	140	·146″	35·0° C.
(*b*)	170	200	140	140–70	·148″	35·0° C.
(*c*)	170	200	140	140	·153″	35·0° C.

(O.P. = Output in c.c. per minute. A.R. = Arterial resistance. Time in this and other tracings, $\frac{1}{5}$″ and $\frac{1}{25}$″.)

PLATE I, FIG. 2.—Exp. 8. 13.6.'21. Dog, 6·6 kilo. Chloralose. Partial auriculo-ventricular block. Pericardium open. Effect of raising the arterial resistance; the inflow was not altered.

	O.P.	M.B.P.	Auricular rate per min.	Ventricular rate.	P-R interval.	Temp.
(*a*)	217	125	124	62	·126″	34·5° C.
	—	185	120	78	complete block	34·5° C.

(M.B.P. = Mean blood-pressure.)

PLATE I, FIG. 3.—Exp. 3. 29.4.'21. Dog, 7·0 kilo. Chloralose. Complete auriculo-ventricular block.

Top line auricle (systole downwards). Middle line B.P. (scale divisions 50 mm. Hg.). Bottom line ventricular movements (systole downwards). Time 1″. Effect of increasing the venous inflow at arrow from 180 to 333 c.c. per minute with arterial resistance constant. Temp. 32·5° C.

PLATE II, FIG. 4.—Exp. 4. 2.5.'21. Dog, 7·0 kilo. Chloralose. Complete auriculo-ventricular heart block. Pericardium half open. Effect of increasing the venous inflow (*a–c*)

	O.P.	A.R.	Auricular rate per min.	Ventricular rate per min.	Temp.
(*a*)	215	60	120	39·5	33·5° C.
(*b*)	830	60	150	37·5 (approx.)	34·2° C.
(*c*)	—	60	150	38·5	34·2° C.

Effect of a rise in arterial resistance immediately after the pericardium had been fully opened (*d–f*):

	O.P.	A.R.	Auricular rate per min.	Ventricular rate per min.	Temp.
(*d*)	215	66	106	18	33·5° C.
(*e*)	—	170	118	72	—
(*f*)	—	170	120	(approx.) —	33·5° C.

PLATE III, FIG. 5.—Exp. 6. 10.6.'21. Dog, 8·4 kilo. Chloralose. Complete auriculo-ventricular heart block. Pericardium open.

(*a*) Small venous inflow: A.R. 170 mm. Hg.; aur. rate 96; temp. 31° C.

(*b*) Effect of increasing the venous inflow to a maximum: A.R. 170 mm. Hg.; aur. rate 100; temp. 31° C.

Effect of reducing both the arterial resistance and venous inflow when both are initially high:

	O.P. per min.	S.B.P.	Auricular rate.	Ventricular rate.	Temp.
(*c*)	660	140	100	Irregular	31·5° C.
(*d*)	86	80	83	41·5	31·0° C.

PLATE III, FIG. 6.—Exp. 7. 10.6.'21. Bitch, 7·8 kilo. Chloralose. Pericardium open. Complete auriculo-ventricular block. Effect of decreasing the arterial resistance:

	O.P.	A.R.	Auricular rate per min.	Ventricular rate per min.	Temp.
(*a*)	375	175	172	55	36·0° C.
(*b*)	350	90	172	92 (approx.)	36·0° C.
(*c*)	—	0	158	38·5	36·0° C.

(*b*) and (*c*) are marred by deflections due to the artificial respiration, shown by arrows.

THE IMPORTANCE OF THE INORGANIC CONSTITUENTS OF THE FOOD IN NUTRITIONAL DISORDERS. I: RICKETS IN PIGS.

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RICKETS is very prevalent among pigs kept in confinement. In some districts the incidence of the disease in litters born in the beginning of winter is as high as 50 per cent. It is thus a cause of very considerable loss to the agricultural community and to the country.

Whether the ætiology of the condition is similar to that of human rickets is not definitely established. The chief gross signs of the disease are, in order of sequence of time, rough lustreless appearance of coat with excessive growth of hair, loss of appetite, slowing of rate of growth, lethargy, difficulty in locomotion, as shown first by a stiff, stilted gait. Later there follows loss of power of the hind legs, tendency to fracture of the ribs and deformities of the long bones. Slowing of the rate of growth usually affects the head less than the rest of the body, so that in the late stages the head often appears abnormally large.

The experiments described here were carried out in the course of an investigation undertaken on account of the economic importance of the subject, to determine what conditions of housing or feeding favour the onset of the disease. Some of the results obtained which seem of interest in connection with recent work on experimental rickets are presented here.

METHODS AND NATURE OF INVESTIGATION.

A feeding experiment carried out here in 1919–20 showed that young pigs in confinement on a diet of wheat offal, grains and potatoes develop the rickets syndrome as outlined above. It was thus possible to arrange a basal diet of these feeding stuffs which would with a degree of certainty produce the condition, and, by the addition of various substances to this diet, to determine what factors prevented the occurrence of the disease.

A consideration of the nature of wheat offal, grains and potatoes suggests (1) deficiency of "fat soluble A," and (2) a lack of correspondence between the mineral matter of the food and the mineral requirements of the animal.

A series of experiments were carried out, in each of which, in a control group of animals, the supposed deficiency of the basal ration in both "fat soluble A" and mineral matter was roughly adjusted, and in the experimental group the deficiency in either "fat soluble A" or mineral matter was allowed to remain, so that the influence of each of these factors in preventing the disease was tested separately. "Fat soluble A" was added in the form of cod-liver oil. The salt mixture used to adjust the mineral deficiency was a rough

imitation of the ash of milk with either additional calcium, to make good the marked calcium deficiency of the basal diet and sodium hydrate to reduce the excessive acidity, or of a calcium-rich mixture of salts supplied in a separate box to which the animal had access. The details are given with the experimental data. Water soluble C was added to all the rations in the form of either lemon juice or swede turnip to avoid the possible complication of deficiency of this factor, though, as a matter of fact, the result of a series of experiments which we conducted in the course of the investigation seem to indicate that the pig is very little susceptible to a deficiency of this vitamin.

In this communication four experiments are briefly described, two showing the influence of deficiency of mineral matter, and two the influence of deficiency of fat soluble A vitamin.

EXPERIMENTAL DATA:

INFLUENCE OF INORGANIC CONSTITUENTS.

Experiment 1.—Two groups each of three pigs two months old were housed under identical conditions in adjoining pens. The pens had an open-air run, with a brick floor. The groups were comparable as to sex and litter, *i. e.* each pig in one group was balanced by a pig of the same sex out of the same litter in the other group.

The basal diet was as follows:

	Proportion.
Oatmeal	100
Bran	125
Blood meal	25
Swede turnip	75
Potatoes	37·5

Marmite, 20 grm. to each group per day.
Cod-liver oil, 30 c.c. to each group per day.

To reduce the proportion of protein in the diet as the animals increased in age, as is usually done in practice in feeding stock, blood meal was replaced by stages by maize meal. Water was always available. The animals were allowed to eat to their appetites.

One group received only the ration. To the other group there was added per pig per day 2 grm. calcium phosphate and 0·25 grm. each of magnesium sulphate, potassium carbonate, ferric chloride and sodium chloride. In addition to the small quantities of inorganic matter added to the food there was always in the pen a box, which was kept full of a mixture of salts, consisting chiefly of chalk, bone meal, rock salt and coal ashes. The mixture was eaten by the animals, and had to be renewed from time to time.

The feeding was continued for four months.

Results.—The animals of the group receiving the salts showed normal growth and no signs of disease. Those of the group receiving only the basal ration began to show signs of malnutrition within the first thirty days. There was lack of lustre of the hair, decreased appetite and slower rate of growth. By the end of the second month the animals had become lethargic, and when forced to move, their gait was stiff and stilted. In the third month deformities of the limbs became evident.

The comparative rates of growth are shown by the increase in weights given in the following table:

TABLE I.—*Increase in Weights.*

	Initial weight. Kilos.	Weight after 124 days. Kilos.	
No. 36	13·2	35·5	Ration only.
No. 37	11·3	43·0	
No. 27	19·4	59·7	
No. 40	11·3	66·0	Ration plus salts.
No. 31	11·9	73·2	
No. 33	19·7	85·0	

The average gain per pig per day was in the "ration only" group 0·25 and in the "ration plus salts" group 0·49 kilos.

On the 124th day No. 27 and No. 31—corresponding animals in the two groups—were killed and the percentage of calcium and phosphorus in the eighth rib and femur estimated:

TABLE II.—*Showing Percentage of Ca and P in Ribs and Femur.*

	Ribs.		Femur.		
	CaO.	PO.	CaO.	PO.	
No. 27	17·1	14·2	29·6	22·8	No extra salts.
No. 31	20·6	16·5	33·4	25·7	Calcium-rich salts.

Microscopic examination of the bones of No. 27 as compared with No. 31 showed some irregularity of the epiphysial line, an excess of osteoid tissue and disorganisation of the zone of proliferation in the cartilage.

Experiment 2.—In this experiment the basal diet was similar in nature to that on which Mellanby (1918) produced rickets in puppies, except that the fat was removed from the milk. Oatmeal and rice were fed in equal proportions according to appetite with an addition of 800 c.c. machine-skimmed milk per animal, *i. e.* about 80 c.c. per kilo weight of the animal at the beginning of the experiment, and 10 grm. sodium chloride per pig per day.

Four groups, each of four seven-week-old newly weaned pigs, were used. The groups as before were comparable with each other as to sex and litter, and the housing conditions were identical.

In comparison with the milk of either the bitch or the sow, Mellanby's basal diet is very poor in protein. Bitch's milk contains roughly, per cent., protein 10, fat 11, carbohydrate 3, and for the sow the respective figures are 7, 5 and 3. All the food-stuffs of the basal diet contain a much lower percentage of protein in proportion to the other energy-yielding constituents than that present in the milk of the species experimented on. To see whether deficiency of protein has any influence, additional protein in the form of blood meal, *i. e.* dried blood, was added to the basal ration of two of the groups to the extent of one-sixteenth of the total weight of oatmeal and rice given.

To the food of one of the two groups receiving the ration only and one of the two receiving the ration plus extra protein there was added per pig per day 20 grm. of the salt mixture used in Experiment 1 plus 5 grm. calcium

carbonate. In addition there was added to every 6 pounds of mixture of rice and meal 80 c.c. of 10 per cent. sodium hydrate—the approximate amount necessary to neutralise the excessive acidity of the ration.

The diets were therefore as follows:

Group I.	Group II.	Group III.	Group IV.
Ration only	Ration + salts	Ration + protein	Ration + protein + salts.

Water was always before the animals but no additional mineral matter was provided.

Results.—During the first fortnight the rate of growth of the two groups receiving the extra protein exceeded that of the other two groups. During the second fortnight the signs of malnutrition noted in Experiment 1 became evident in Group III, and one of the animals died on the thirty-third day of the experiment. The *post-mortem* examination showed anæmia and patches of inflammation and adhesions in the pleura of the lungs. In the third fortnight the signs of disease noted in Group III appeared in Group I. Towards the end of the second month two of the three remaining animals of Group III were hardly able to walk and were reluctant even to stand. The remaining animal of the group had acquired the habit of drinking the urine of its companion. It was the last to show signs of disease.

The following table shows the average gains in weight in the different groups during the first 30 days. Owing to the death in Group III the averages for the second month during which the experiment was run are not comparable.

TABLE III.—*Average Gains in Weight in Different Groups.*

Groups.	Initial weight. Kilos.	Gain in weight. 1st 15 days.	Gain in weight. 2nd 15 days.
I. Ration only	9·8	3·2	3·4
II. Ration + salts	9·8	3·1	4·4
III. Ration + protein	9·7	3·6	2·5
IV. Ration + protein + salts	9·8	3·5	4·9

On the sixty-ninth day one of the animals of Group III was killed. The following table shows the percentage calcium in the eighth rib compared with that found in a healthy animal of the same age.

TABLE IV.—*Showing Percentage Composition of Dried Rib (Eighth).*

Organic matter.	Ash.	CaO.	PO.	
58·3	41·7	21·7	17·3	No. 99 of Group III.
46·6	53·4	28·0	22·0	Normal animal same age.

Microscopic examination of the bones showed the same pathological characters as those found in Experiment 1, but the condition was less advanced.

No sign of rickets appeared in either of the groups receiving the extra salts. The signs of the disease usually appear first, as Mellanby (1918) has pointed out, in the most rapidly growing animals. In Groups II and IV the rate of growth was rather above the normal. One of the animals in Group II

during the last ten days of the experiment increased in weight 0·97 kilos per day and one in Group IV 0·82 kilos. In these groups there was no sign of malnutrition, though the diet, with the exception of the inorganic matter, was exactly similar to that of Groups I and III, where the gross evidence of the disease appeared within thirty days.

INFLUENCE OF FAT SOLUBLE A (COD-LIVER OIL).

Experiment 3.—In this experiment ample inorganic matter was given to both groups, and one group was given additional fat soluble A in the form of cod-liver oil, so that the influence of a relative deficiency of "fat soluble A" was determined in the other group.

Two groups each of three animals all out of the same litter were given the following ration:

	Proportion.
Oatmeal	100
White bread (replaced after first month by maize)	200
Cod muscle	200
Swede turnip	100
Marmite, 20 grm. per day.	
Salt mixture as in Experiment 1.	

Access was allowed to water and to a salt box as in Experiment 1.

Cod muscle was used as the supply of protein, because it contains no fat soluble A (Medical Research Committee, 1919).

To one group was given 10 c.c., rising to 20 c.c. cod-liver oil, and to the other 10 grm., rising to 20 grm. rendered lard, which is stated to contain little or no fat soluble A.

Results.—So soon as the experiment commenced all the animals of both groups began to suffer from diarrhœa. The condition was more marked in the lard group. One of the animals in this group (No. 23) developed distension of the abdomen and a rough "staring" coat—a sure indication of malnutrition. Boiling the fish produced no improvement. At the end of the first month maize meal was substituted for bread and the diarrhœa stopped in both groups within 48 hours.

During the subsequent period of the experiment, which was continued for 132 days, no signs of disease appeared in either of the groups. At the close of the experiment, however, the general appearance of the cod-liver oil group was better than that of the lard group, in so far as the coats were more silky and glossy—a sure sign of health—and the increase in weight was greater.

TABLE V.—*Showing Increase in Weight in Kilos.*

	Beginning of experiment.	105 days.	132 days.	
No. 14	11·7	69·0	80·8	Cod liver oil.
„ 15	12·6	70·0	—	
„ 16	13·0	75·1	95·8	
„ 17	10·9	61·3	69·5	Lard.
„ 20	12·1	60·8	70·7	
„ 23	13·5	68·2	83·2	

Table V shows the respective increase in weight (1) on the 105th day, when one of the cod-liver oil group had to be killed owing to its having received an accidental traumatic injury and (2) on the 132nd day—the end of the experiment.

No. 23 of the lard group, the animal which had shown the signs of malnutrition in the earlier part of the experiment, was continued on the diet until the 140th day, when it was killed. During the last 30 days of this period it had shown an average increase in weight of 0·61 kilos per day. The post-mortem examination showed no abnormality. The epiphysial line was regular and clearly defined, and the bones were of the usual degree of hardness. No. 20 was also killed and showed nothing abnormal in the post-mortem examination. No. 17, a sow, was kept for breeding purposes. She was turned out to graze and mated 34 days after the close of the experiment and had a litter of eight healthy pigs.

Experiment 4.—Two groups each of four seven-week-old pigs comparable as to sex and litter and in similar housing were given the following basal diet :

	Proportion.
Bran	100
Oats (crushed)	100
Blood meal	8
Salt mixture as in Experiment 2.	
50 c.c. lemon juice per group.	

The proportion of blood meal was reduced by stages as the animal increased in weight and entirely omitted at the end of the third 30 days.

There was added to one group 10 c.c. cod-liver oil and to the other 10 c.c. linseed oil per pig per day. The linseed oil, which is deficient in A factor (Medical Research Committee, 1919), was added to balance as far as possible the caloric value and the physical properties of the cod-liver oil.

The experiment was continued for 120 days.

Results.—None of the animals throve during the first month. The gains in weight were below the normal and the coats had not the silky, glossy appearance that denotes perfect health in the pig. The diet was probably too harsh for newly-weaned pigs. Both groups improved in condition as the experiment proceeded. No signs of disease appeared in any of the animals.

During the first 60 days the linseed oil group grew faster than the cod-liver oil group. Thereafter the reverse was the case. The following table shows the increase in weight per pig during the successive periods of 30 days :

TABLE VI.—*Average Increase in Weight per Pig per Day in Kilos.*

	1–30.	31–60.	61–90.	91–120 days.
Cod-liver oil group	·086	·238	·434	·538
Linseed oil group .	·134	·266	·403	·453

In Experiments 1 and 2 definite signs of the disease appeared during the second month. As this experiment had been continued for 120 days and the animals had passed the stage when rickets usually develops and all appeared in perfect health, the experiment so far as rickets is concerned was considered finished.

One animal out of each group was killed and examined. No abnormality was discovered in either. The percentages of calcium and phosphorus in the femur are given in the following table:

TABLE VII.—*Showing percentage of Ca and P in Femur.*

	CaO.	P_2O_5.	
No. 106	34·68	26·24	Cod liver oil.
No. 108	34·21	26·58	Linseed oil.

SUMMARY OF RESULTS.

In Experiment 1, on a diet in which the energy-yielding constituents are derived chiefly from bran and oatmeal, and which contained an abundance of the three well-known accessory factors, the signs of rickets appeared during the second month. In animals of the same sex and litter, housed under identical conditions and receiving the same diet, but with the addition of a mixture of inorganic material consisting chiefly of calcium salts, no signs of the disease were apparent at the end of four months, by which time the animals had passed the stage where the disease usually appears.

In Experiment 2, on a diet of oatmeal and rice with 80 c.c. skimmed milk and 1 grm. sodium chloride per day per kilo weight of the animal at the beginning of the feeding period, the signs of the disease developed and were well marked by the end of the second month. The addition of a calcium-rich salt mixture to a control group under the same condition of housing and feeding prevented the development of the disease.

In the group receiving the protein-rich diet the growth at first was more rapid and the pathological condition developed earlier. This is in accordance with Mellanby's (1918) observation on rickets in puppies, that the most rapidly growing puppies are most susceptible to the disease.

In Experiments 3 and 4 the diets were purposely designed to contain a minimum of accessory factor "fat soluble A," and certainly contained no more of this than is present in the rations of animals where the disease is occurring in practice. Both the experimental and the control groups had, however, a luxus consumption of calcium-rich mixture of salts. Though the monotonous diet was continued in the one case for 120 days and in the other for 140 days, the rickets syndrome did not appear.

In both of these experiments, though the groups receiving the diet deficient in fat soluble A did not develop rickets, the growth there was less vigorous than in the group receiving the cod-liver oil. Further, in Experiment 3 the health of the animals, as evidenced by the condition of the coat and their activity and liveliness, was better in the group receiving cod-liver oil than in the one receiving the lard.

DISCUSSION OF RESULTS.

In connection with this investigation, papers will shortly be published dealing with the mineral requirements of growing animals, and the physiological importance of the ratio of the inorganic constituents of food-stuffs to each other. The results presented here need therefore only be discussed briefly.

Grains, and food-stuffs derived from them, are, in general, characterised by a deficiency of calcium, and by the fact that the ratio of magnesium to calcium, of phosphorus to calcium and of potassium to sodium is very high. The percentages of these minerals in the dry matter of the chief feeding stuffs used in the present experiments are as follows (Forbes, 1913):

	K.	Na.	Ca.	Mg.	P.
Bran	1·46	0·22	0·14	0·59	1·23
Oats	0·46	0·18	0·11	0·13	0·43
Rice	0·32	0·04	0·01	0·03	0·10
Potatoes	1·55	0·06	0·03	0·33	0·27

It may be assumed that the proportions in which these minerals are present in milk are those best suited for the growing animal. In the ash of the sow's milk, the percentages as quoted in König's tables (1889) are as follows:

K.	Na.	Ca.	Mg.	P.
6·22	6·73	39·22	1·77	37·21

Compared with this composition of ash, there was in the basal diets used here a deficiency of calcium, and a relative excess of magnesium, potassium and phosphorus. The addition of calcium-rich salts to the ration used in these experiments would tend to correct the mineral deficiency.

The beneficial effects of calcium for farm stock suffering from nutritional disorders affecting the bones has long been known. A review of the literature to 1912 is given by Hutyra and Marek (1913).

With regard to work on the importance of the proportions in which the inorganic constituents are present, Ingle (1908) has pointed out the evil effects that may follow a prolonged diet in which there is a great excess of phosphorus in proportion to calcium, and has suggested that the well-known "bran disease," or "miller's horse rickets," is due to this cause. Zuntz (1912) has shown that certain fodders that cause bone-lesions in horses are injurious in proportion to the excess of potassium to sodium in the ash.

Hart and McCollum, with their co-workers, in the course of their valuable work on deficiencies of the different grains have shown that the deficiency of grains in mineral matter is so great that a diet consisting exclusively of grains will not support growth, even though any other deficiencies that may be present are made good. Hart, Miller and McCollum (1916) produced a pathological condition in pigs which, from the description given, appears to be similar to that which we have been investigating. They indicate that three factors may be involved in the production of the disease, viz. (1) deficiency of mineral matter, (2) deficiency of fat soluble A, and (3) toxicity of wheat products. In our experiments the condition did not occur when the amount and ratios of the different inorganic constituents were adjusted, and we are inclined to believe that the frequently noted toxic influence of wheat offal is due to the composition of its ash, which, with other deficiencies, shows a marked excess of phosphorus to calcium. Their own results seem to support this view. They state—"where alfalfa constituted 20 to 25 per cent. of the ration and middlings 20 per cent., the toxicity of the middlings was overcome, the inadequacy of the

grains disappeared, and the animals remained sound and vigorous"; and again, "with commercial meat scraps as supplements to a corn, middlings, oats, oil mixture as previously described, we have also had splendid success in obtaining normal growth and sustained vigour." Both these additions to the diet tended to adjust the mineral matter. The meat scraps used by them contained 6·5 per cent. calcium, and alfalfa is very rich in calcium as compared with phosphorus. Forbes (1913) gives the percentage composition of the dry substance of alfalfa as—

K.	Na.	Ca.	Mg.	P.
0·83	0·49	1·13	0·40	0·24

An abundant supply of calcium of course does not correct the mineral deficiency in every case. Prominence is given to it here because, with regard to mineral matter, deficiency of calcium was the marked feature in the diets used in the experiments and was probably the chief factor in the production of the disease. Shipley, Park, McCollum and Simmonds (1921) have shown the importance of phosphorus in the prevention of rickets, and Sherman and Pappenheimer (1921) have shown that in deficiency of both calcium and phosphorus osteoporosis occurs; with the addition of calcium lactate rickets occurs, and with the further addition of potassium phosphate rickets is prevented. It is most probable that the presence of a sufficient amount of any essential inorganic constituent need not necessarily prevent rickets. To ensure normal growth and health there must be not only a sufficiency of each of the essential inorganic constituents, but the ratios of these to each other must be adjusted to the needs of the animal, or at least must not show such a wide divergence from the physiological optimum that the excess of any of them is greater than the animal can deal with without upsetting the normal metabolic processes. Unfortunately, exact information as to what excess of the different minerals can be tolerated is not available.

The influence of fat soluble A can be briefly discussed. It is obvious that an abundance or relative deficiency of it need not necessarily prevent or produce rickets. The results give no support to the suggestion of the Cambridge school that rickets is due to a deficiency of an accessory factor probably identical with fat soluble A. They are in an agreement rather with the work of Noël Paton and Watson (1921), which shows little or no correlation between rickets and any substance associated with animal fats.

It has been already shown by Zilva, Golding, Drummond and Coward (1921) that pigs can continue to grow for at least 70 days on a diet with a low content in fat soluble A. In these present experiments the small amount of fat soluble A in the diet used has enabled growth to proceed over a period of 140 days, during which time the animals increased in weight by over 500 per cent. On the other hand the well-recognised value of cod-liver oil compared with either linseed oil or lard in maintaining health and promoting growth is shown even in the pig, which evidently is not so susceptible to deficiency of fat soluble A as the rat. It is possible that the beneficial effects of certain fats may in part at least be associated with their influence on the assimilation and utilisation of inorganic substances. That is being investigated here.

Since the above paper was prepared there has appeared a communication by Korenchevsky (1921) dealing with the influence of deficiency of calcium and of fat soluble A in producing rickets in rats. He shows that rickets occurs most readily when the diet is deficient in both calcium and fat soluble A. The deficiency of calcium alone, however, produces a change in the skeleton resembling rickets.

NOTES.

(1) The experiments described here and the conclusions drawn from them were dealt with in a paper read to the Agricultural Section of the British Association Meetings at Edinburgh, September 13th, 1921.

(2) We wish to express our indebtedness to Dr. G. M. Duncan for carrying out the pathological work in connection with these experiments, and to Dr. Marion B. Richards and Mr. A. D. Husband for the chemical analysis of the bones.

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A COMPARATIVE STUDY OF NORMAL AND MALIGNANT TISSUES GROWN IN ARTIFICIAL CULTURE.

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One of the easiest methods to obtain comparative biochemical information as to the metabolism of both normal and malignant cells would be to treat such cells as bacteria and grow them in suitable media outside the body. The present work is an attempt to study cells from this point of view and to arrive at a satisfactory artificial medium in which they may be cultured indefinitely. Carrel and Burrows (1912) and others have shown that cells may be grown for many generations in certain media. The great majority, however, of the American workers have used plasma as a culture medium either alone or with the addition of various extracts. Apart from the technical difficulties of growing tissues in such media, plasma is very unsatisfactory when accurate biochemical information is required. Slight differences in various samples of plasma often make great differences in growth, and it is at all times very difficult to control. Even for histological study plasma growths leave much to be desired, as it is often found that the plasma retains the stain quite as tenaciously as the cells themselves. Before discussing artificial culture media, it is necessary to consider the features of growth in plasma of embryonic tissues, adult tissues, and tumours.

The whole of our own work has been carried out with either rat or mouse tissues, and mouse tumours in rat plasma. Most of the American workers have used bird plasma, but as it was thought desirable to use a plasma differing as little as possible from that of the animals from which the tissues were taken, rat plasma was chosen as being specially suitable. It has been found possible considerably to simplify the usual technique for obtaining such material. A hollow platinum needle such as is used for the transplantation of tumours is fused into a glass tube some 20 cm. in length and about 5 mm. internal diameter. This is thoroughly coated with paraffin wax by immersion in a bath of equal parts of paraffin of M.P. 45° C. and 56° C. When required for use the tube is lifted out and quickly drained, air being blown through in order to keep the needle clear. The rat is anæsthetised with ether and the thorax quickly opened with aseptic precautions; the heart is then steadied with forceps and the needle thrust into the right ventricle. The blood is allowed to flow into the paraffined tube till the flow slackens. The needle is then withdrawn

and the contents at once run into paraffin-coated sterile glass centrifuge tubes and centrifuged at high speed for three minutes. I have found the type of tube shown in Text-fig. 1 to be the most convenient pattern for the work. The tubes should be thoroughly cooled before the blood is run in and it is best to surround them during centrifuging with ice and salt mixture. The supernatant plasma is removed with a paraffined Pasteur pipette and is again centrifuged in the cold for one or two minutes. This is necessary to separate the blood-platelets as far as possible and so delay clotting. About two-thirds of the resulting plasma is next pipetted off with a paraffined pipette and is placed in a sterile paraffin-coated glass tube, which is kept in a wider tube packed in ice and salt. The tissues to be cultured are removed from the animal with strict aseptic precautions, and are transferred to sterile glass capsules containing a little sterile Ringer's solution. There is no necessity to keep the tissues at 37° C. prior to culture; in fact they may be stored in the ice-chest, and will still give growths after five days. When required for culture the organ selected, *e. g.* heart, is cut up into small pieces with iridectomy knives and the pieces are kept moist by a few drops of Ringer's solution. A piece is placed on a sterile cover-glass, and is then covered with plasma which is added from a sterile paraffined Pasteur pipette. The plasma clots in the space of a minute or two and the cover-glass is then placed over a well slide and is sealed with paraffin wax. It is then incubated at 37° C. In order to reduce the chances of infection to a minimum it is best to carry out the culturing under cover of a glass case, which can be flamed immediately before use. Subculturing is performed by raising the cover-glass by means of a safety-razor blade, cutting carefully round the growth with iridectomy knives, taking especial care to free it from all traces of plasma. The fragment is next washed quickly in sterile Ringer's solution, and divided if necessary, each piece being planted into fresh plasma as already described.

FIG. 1.—Centrifuge tube for collection of plasma.

All tissues do not behave alike when grown in plasma in this way. The differences can best be brought out by a short description of the results with mouse embryo heart, adult mouse heart, and a rapidly growing mouse sarcoma —37 S. of this laboratory. In 24 hours at 37° C. the embryo heart shows growth all round the edges of the original fragment, the growth consisting of both heart-cells and connective tissue. The adult heart shows no growth whatever either of connective-tissue cells or heart-cells, whilst the sarcoma shows first a circular area of liquefaction round the original fragment with extensive growth around this, in the form of a ring (Plate I, fig. 2*a*); such ring-shaped growths are very characteristic of sarcoma grown in rat plasma. These growths have been frequently described and pictured by several authors, but no explanation of this arrangement of the cells has been advanced. We have observed that cultures of this type show an early liquefaction of the fibrin network immediately surrounding the implanted fragment. Contraction of the peripheral portions of the fibrin network will of necessity lead to the circular

arrangement of the growing cells. The embryo heart continues to grow for several days and may beat actively; the adult heart as a rule shows no signs of growth at any time, but occasionally after a lag of about a week may show slight growth in a very small percentage of cases.

The sarcoma begins to degenerate in 48 hours and unless subcultured dies in from two to three days. Provided it is subcultured at intervals, the embryo heart will live and continue to grow for as long as forty days, but growth finally ceases. The sarcoma, as already stated, degenerates very rapidly after the second day. Repeated subculture every day will, however, give vigorous growths for a month or more, when, as in the case of embryo heart, the cultures die out. Inoculations of such repeatedly subcultured growths into mice give tumours in every case, showing the tumour-cells to be alive and unaltered biologically. Such a process of growth as occurs with embryo heart in a plain plasma medium may be considered typical of all normal embryonic tissues, as I have found all those studied to behave in a similar manner.

From the behaviour of the 37 sarcoma it seemed probable that some toxic substance was elaborated which caused the very early degeneration. A number of plasma cultures were accordingly taken, and both plasma and growth were ground up with sterile sand and a very small quantity of distilled water; this emulsion was then centrifuged and the clear supernatant fluid pipetted off. A series of 12 cultures of embryo heart and 37 S. were put up in a plasma medium containing $\frac{4}{5}$ plasma and $\frac{1}{5}$ of the 37 S. extract. Along with these cultures a further series of heart and 37 S. cultures were prepared containing $\frac{4}{5}$ plasma and $\frac{1}{5}$ distilled water. The embryonic heart grew in every case in both media. The sarcoma grew in every instance in the plasma diluted with distilled water, but failed completely in those fragments immersed in plasma diluted with the extract of 37 S. cultures.

It is evident that 37 S. in culture produces some substance which is toxic to itself and can inhibit growth *in vitro*. This substance is evidently not toxic to heart. Heat at 60°C. for half an hour destroyed its toxicity. Most of the tumours tested showed varying degrees of toxicity but not to the same extent as did 37 S.

It will be observed from these experiments that the various tissues and tumours can be grouped into three divisions, viz. embryonic tissues, which grow vigorously in a plain plasma medium ; tumours which also give growth, but not to such an extent as do the embryonic cells; and adult tissues which show little or no growth. This point will have to be discussed in greater detail when considering the growth of tissues in artificial media. The addition of various amino-acids (glycine, alanine, valine, arginine and histidine) to rat plasma gave in most cases a somewhat greater initial growth than was obtained in plain plasma. Microscopic examination of such growths showed that the majority of the cells were undergoing amitotic division, very few mitoses being observed. Plasma rendered hypotonic by the addition of distilled water gave an exactly similar effect. I believe that this represents a degenerative phenomenon, as cultures made in this way die out at an earlier date than do plain plasma cultures, and there seems to be little doubt that the cells are quite unable to use the amino-acids as food

substances—in fact the continued presence of these acids seems to be toxic to the cells.

It is extremely desirable to be able to dispense with plasma as a culture medium in order that the conditions may be more rigidly controlled. Lewis (1920) showed that a certain amount of growth takes place in Locke's solution containing 0·25 per cent. glucose and 10 per cent. of chicken bouillon. Such growths appear to have been poor, however, and probably represent little more than survival of cells. It was found that better growths could be obtained with this Locke-Lewis solution if the calcium chloride and the sodium bicarbonate were steamed separately and added before use. Cultures in such solutions, however, tend to die out at an early date, and cannot be subcultured indefinitely, and are never equal to growths obtained in plasma. After many trials the following solution, the inorganic constituents of which are nearly identical with those of plasma, was found to be satisfactory:

NaCl . . .	0·900
KCl . . .	0·042
$NaHCO_3$ . . .	0·020
$CaCl_2$ . . .	0·020
$CaH_4(PO_4)_2$ . .	0·010
$MgHPO_4$ . . .	0·010
H_2O . . .	100

The salts used in the preparation of this solution must be pure. It will be found best to make up stock solutions of the various salts ten times as strong as the final concentration. Ten c.c. of the NaCl, KCl and $MgHPO_4$ solutions are then pipetted into a flask and 40 c.c. of distilled water added. The calcium solutions and the bicarbonate are steamed in three separate flasks, and just before use 10 c.c. of each is pipetted into the mixture. The solutions should not be autoclaved. The best results are got by steaming for not more than ten minutes. The finished solution should have a faint bluish opalescence. If a precipitate is formed such as occurs when the fluid is autoclaved it is useless. The pH of the fluid should be 7·4, since growth only appears to occur within very narrow limits of this value. Examination of the fluid so prepared by dark-ground illumination shows very fine particles in active movement. These disappear on the addition of a weak acid, such as acetic acid. In this solution I have obtained as good growths of embryonic tissues as in plasma, for a few generations. The same remarks, however, that were made regarding plain plasma growths apply here, viz. that growth gradually ceases and tissues ultimately die. No growth of adult tissues could be obtained in such a fluid, and it was noticed that although embryonic tissues grew well, tumours were far more difficult to grow in such a fluid than in plasma. The addition of glucose to the fluid gave no better results. As such fluids contain no nitrogen the addition of rat serum was tried, but the resulting growths were no greater than were obtained without it. The addition of embryo extract was also tested. The extract was prepared by finely mincing mouse or rat embryos in a small quantity of the above saline solution, and freezing and thawing the mass two or three times so as to disintegrate the cells as much as possible, and then

centrifuging till a clear or faintly opalescent fluid was obtained. Cultures were put up containing 2 parts of such an extract with 3 of the saline solution, and very rapid and vigorous growths were obtained both of embryonic tissues and tumours. These cultures could be subcultured apparently indefinitely, and increased greatly in bulk. Growths of adult tissues could also be obtained, though with far greater difficulty. Once growth started, however, it was easy to keep it growing in subculture.

It seems evident from these experiments that for the continued growth of tissues outside the body at least two factors are necessary—a saline solution in which the calcium salts are probably in the colloidal state, and some substance or substances contained in an extract of embryonic cells. Indeed Carrel and Ebeling (1921), in a recent paper, appear to consider that the indefinite multiplication of cells in a medium is due entirely to substances contained in embryonic juice. This conclusion is supported, not only by the above experiments, but by the fact that continuous growth cannot be obtained in plain plasma cultures unless embryonic extracts are added to them. Heat at 60° C. for an hour seems to impair greatly the efficacy of the extracts. Whatever the substance or substances in embryo extracts are, it appears unlikely that they are merely a product of all rapidly-growing tissues, as tumour extracts cannot replace embryo extract. It was further found that proliferating connective tissue furnished an extract which was inert. This tissue was obtained by inoculating diatomaceous earth subcutaneously into rats. The extraction was made in the manner already described for making embryo extract. The plasma of a pregnant animal was tried, but it also could not replace embryo extract, so that, whatever the substances are, they are evidently not present in the maternal circulation. As has already been stated, a certain limited amount of growth can be got from both tumours and embryonic tissues in a plain plasma medium. It seems probable, therefore, that in such cases where limited growth occurs without the addition of extract, it must be due to substances carried over with the original fragment. When this is used up growth ceases. Tumours would, on this view, contain some of the necessary substances for growth, but evidently not in anything like the amount that embryonic extracts do, and, judging from these experiments, the adult tissues of the mouse and rat must contain little or none. A point arises in this connection of great interest, viz. that although adult mouse tissues are most difficult to cultivate, yet tumours will grow comparatively easily, and show in most cases abundant growth of the stroma as well as of parenchyma. The stroma growth is, of course, derived from the host, and it therefore appears that the tumour has a very definite action on the connective tissues of its host, enabling them to grow in a similar manner to embryonic tissues outside the body.

Complete loss of differentiation does not appear to be a necessary consequence of successful culture *in vitro*, especially in fluid media. Thus, heart may be grown for generation after generation, all traces of the original fragment having disappeared, and the growth consisting of a thin, rapidly growing, pulsating sheet of cells. These cells if suitably fixed and stained (alcohol acetic or Fleming fixation, followed by iron-hæmatoxylin) show typical myofibrillæ. Most of the American observers have described heart

growths as consisting of fibroblasts. Lake (1916), who observed pulsation of individual cells, was the first to suggest that these were really muscle-cells. There can be no doubt that this is the correct view, the cells in most cases being typically striated as shown in Plate II, fig. 3*a*, and Plate III, fig. 3*b*. It has been stated that no pulsation occurs in heart cultures till a certain amount of tension has been obtained by contraction of the medium. Cultures in this liquid medium, however, frequently show active pulsation when the fragment is floating free, so that external tension is out of the question.

Cultures of embryo skin present interesting features. Growth spreads out from the original fragment as a thin sheet of shorter or longer spindle-cells showing active mitosis (Plate IV, fig. 4), and growth continues in this form

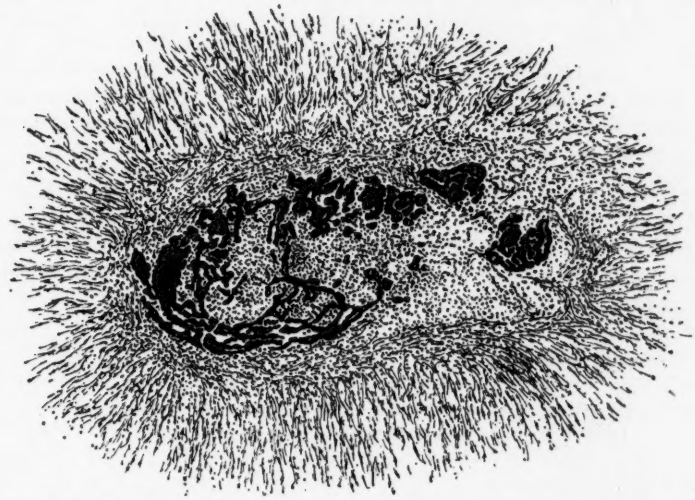

FIG. 7.—Five-day culture of mouse carcinoma 206, showing acinous type of growth of parenchyma with abundant stroma growth. × 50.

if sub-cultured every three or six days. If, however, the culture be left to itself for five or six days, keratinisation begins (Plate IV, fig. 5). Cultures of mammary carcinoma such as strain 206 of this laboratory show at first only a rapid growth of stroma. In forty-eight hours, however, the parenchyma commences to push through the stroma in the form of finger-shaped processes (Plate V, fig. 6). These gradually spread out and in later subcultures show an acinous appearance (Text-fig. 7). In some cases the stroma tends to die out after the first subculture and a pure growth of parenchyma is obtained.

All such cultures show that there is a peculiar relationship between the fibroblasts and the epithelial cells. For example, in cultures of carcinoma 206, where an abundant growth of stroma as well as of parenchyma is obtained, the parenchyma shows an acinous type of structure in the later subcultures as growth progresses. If, however, this tumour be subcultured very frequently,

from the first the growth of parenchyma is free from stroma. Such a growth merely forms a mass of undifferentiated cells with no tendency to form acini. Similarly while skin is growing with a large amount of connective tissue keratinisation is frequent, but when, as occasionally happens, the skin grows out as a sheet away from the connective-tissue cells, no keratinisation occurs and growth continues as a mass of rapidly dividing spindle-shaped cells. The same thing applies to heart. Where the heart-cells grow in proximity to connective-tissue cells they show typical myofibrillæ, but if the connective-tissue growth fails, or the heart-cells grow away from the connective tissue, they appear merely as spindle-shaped cells and myofibrils are absent, though the beat does not seem to be affected by such a change, at all events for some generations. Tissues growing badly, either through the medium being not quite suitable or through insufficient subculture, commence to show degenerative changes. This in its earliest form shows itself as an accumulation of granules within the cytoplasm till the cell becomes packed. These granules give all the reactions of fat, and if cells be examined in the early stages of degeneration, the fatty granules may be seen to arise from a transformation of the mitochondria, in the same way as has been described by many authors in normal and pathological fat formation.

DISCUSSION.

The continued growth of embryonic tissues is easily obtained outside the body in suitable coagulated or liquid media. It is notorious that when transplanted into living animals continued growth does not occur. At the most, survival of cells after temporary multiplication is obtained. The cells of malignant new growths present, at first sight, a remarkable contrast. Continued growth is easily obtained in the living animal, but *in vitro* early degeneration and death necessitate frequent subculture if even a limited growth is to be achieved. These experiments show that this early death of the cells is due to elaboration of toxic substances, which quickly reach a lethal concentration. In addition to a proper colloidal condition of the calcium salts, which is essential in the fluid medium replacing the complicated coagulated plasma, previously used almost exclusively for this kind of work, a substance or substances not heretofore found except in extracts of embryonic tissues is necessary for continued growth of all cells *in vitro*. It has not been demonstrated in rapidly growing tumours nor in regenerating tissues of the adult so far as our experience has gone. It is not possible at present to say whether this growth-promoting substance plays a part in the growth of normal adult tissues (*e.g.* in regeneration) and of tumours *in vivo*, or whether its importance in growth *in vitro* is mainly a consequence of the technical environmental conditions involved.

The experiments bring out very clearly the close biological relations subsisting between tumour parenchyma and stroma. The stroma of a transplanted tumour, although derived from the adult host in which the tumour is growing for the time being, behaves in culture like an embryonic tissue rather than like that of an adult. The presence of stroma elements, although not necessary for the growth of the parenchyma *in vitro*, exerts a profound

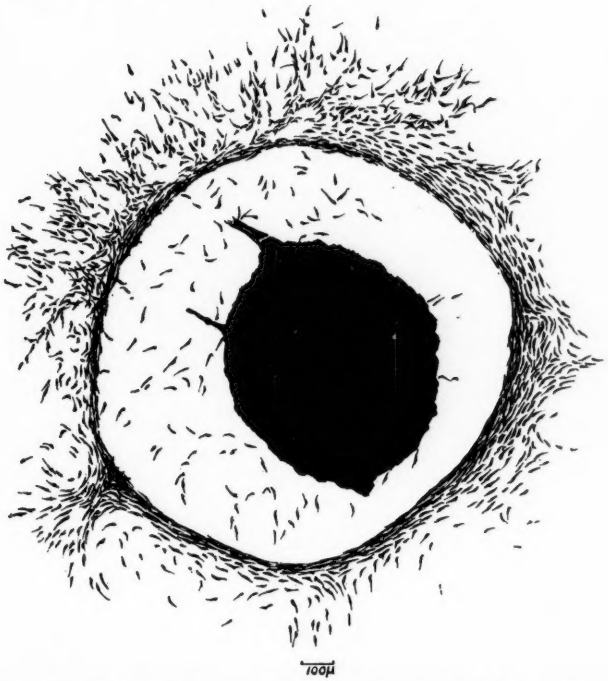

FIG. 2 *a*.—Plasma culture of 37 S. sarcoma, 24 hours' growth, showing liquefaction of plasma round original fragment and typical ring-shaped growth of the sarcoma cells.

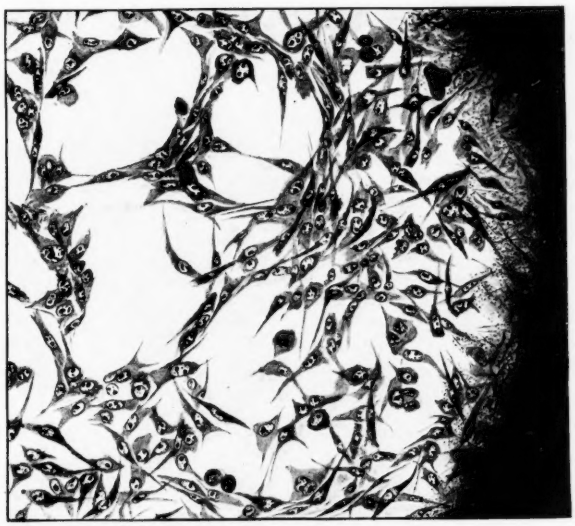

FIG. 2 *b*.—Portion of sarcoma culture in fig. 2 *a*, showing spindle cells. × 250.

Plate I. Drew. Normal and Malignant Tissues in Culture.

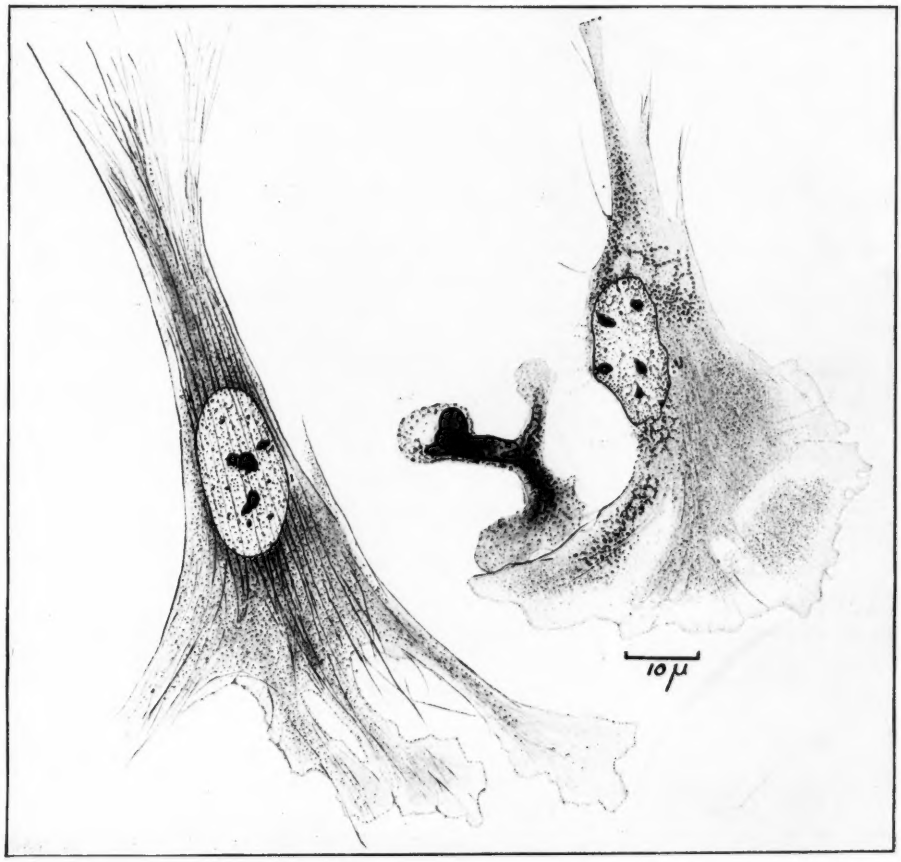

FIG. 3 *a*.—Isolated cells from culture of embryo mouse heart in saline medium, showing myofibrillæ.

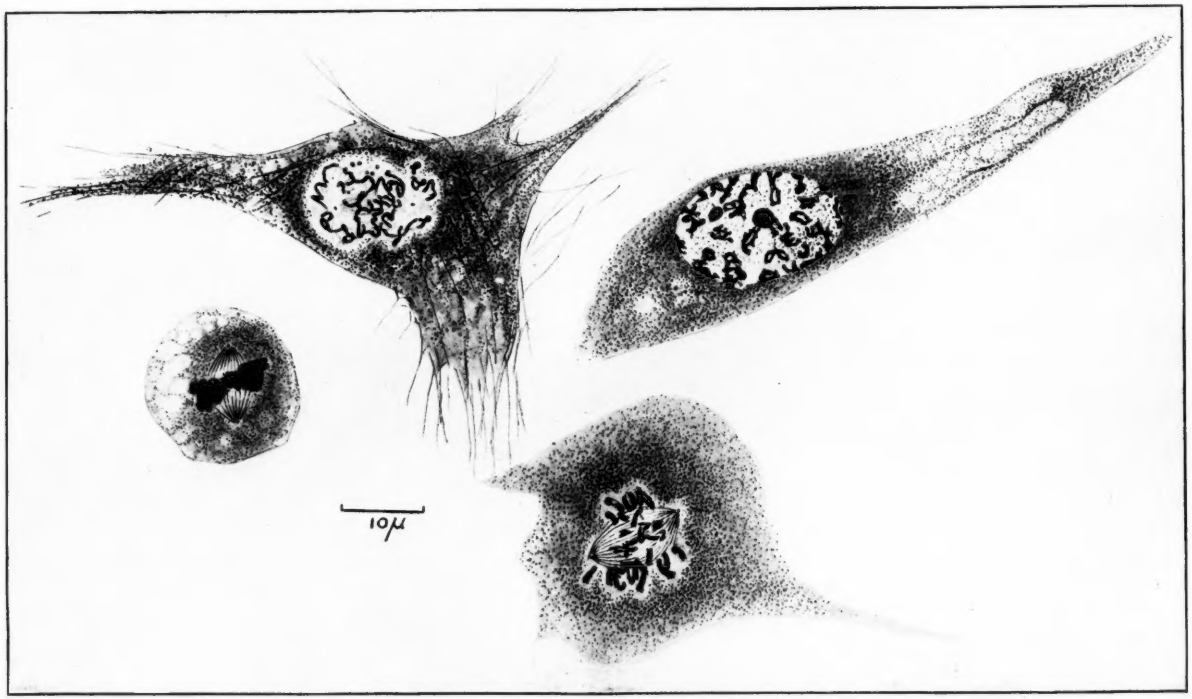

FIG. 3*b*.—Isolated cells from culture of embryo mouse heart in saline medium, showing mitotic figures.

Plate III. Drew. Normal and Malignant Tissues in Culture.

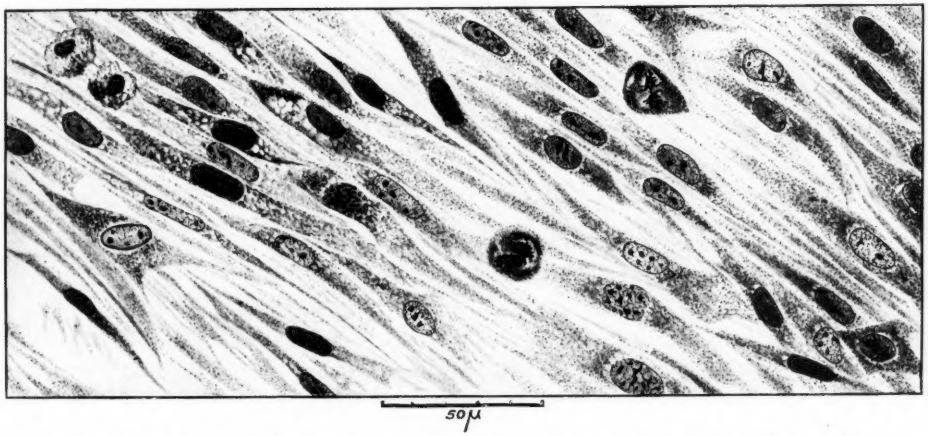

FIG. 4.—Young culture of embryo mouse skin in plasma medium, showing spindle-celled type of growth.

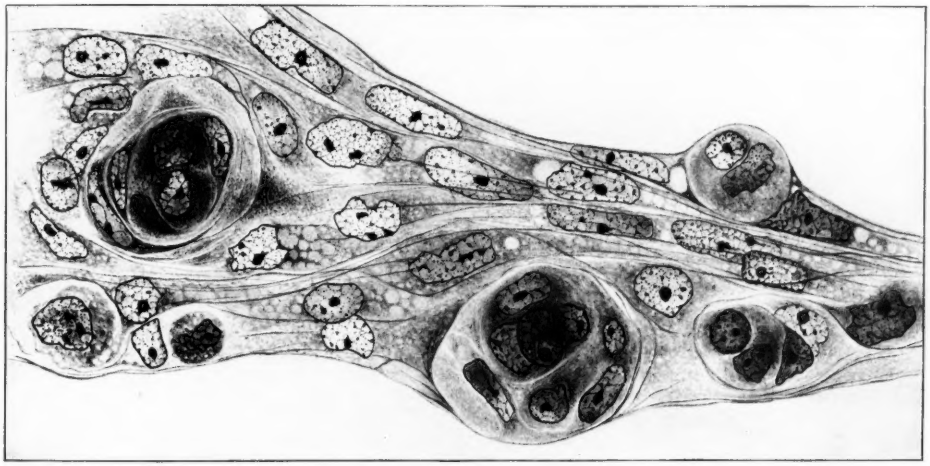

FIG. 5.—Plasma culture of embryo mouse skin, showing commencing keratinization. × 1500.

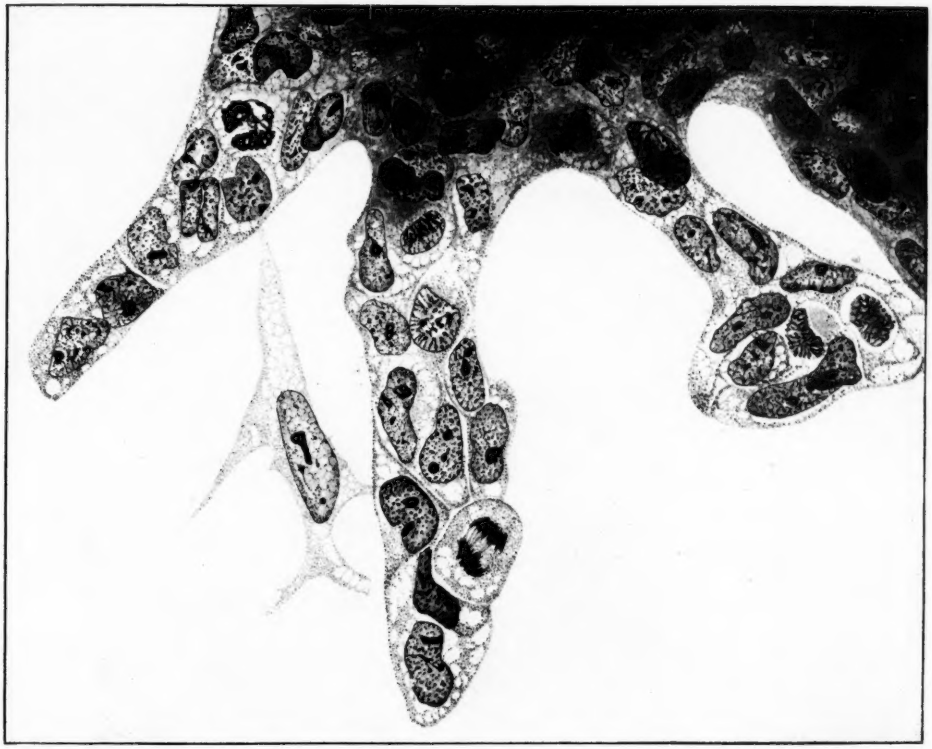

FIG. 6.—Young culture of mouse carcinoma 206, showing growth as finger-shaped processes. × 1500.

influence upon it, and in particular partly determines the degree of differentiation reached by the tumour-cells. The analysis of the factors responsible for these relations of cells to their environment is being made the subject of continued study.

SUMMARY.

Continued growth of normal and tumour-cells *in vitro* is only obtained in the presence of substances so far only found in embryonic extracts. A salt mixture in which the calcium is present in the colloidal state can, with the addition of embryo extract, replace plasma for this work. The degree of differentiation shown by cultures of normal and malignant tissues is partly conditioned by the accompanying growth of the stroma. The stroma behaves in culture like embryonic tissue rather than like the adult tissues from which it is derived.

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[All the figures have been drawn by Mr. W. Pilgrim, of the laboratory staff.]

ON UREA TESTS OF RENAL FUNCTION.

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In the course of some combined clinical and laboratory work on diabetes mellitus and certain glycosurias* I am attempting to gauge the condition of the kidneys by simple renal function tests. For this purpose the urea tests, among others, are being used. Partly as a result of these experiments and partly from the experience gained from the routine examination of hospital patients with kidney disease several facts of importance have come to light. It is felt that these points may be of interest to those more directly studying renal efficiency. In estimating the concentration of urea in the urine (hypobromite method) and in the blood (urease method) the techniques outlined by Maclean and de Wesselow (1919, 1920) were followed. Blood-urea estimations were always performed in duplicate and the mean taken.

THE PROVOCATIVE UREA CONCENTRATION TEST.

For details of this test the reader is referred to the publications of MacLean and de Wesselow (1919, 1920). In the great majority of 400 cases the results have been most helpful. But occasionally an interpretation of the figures has been difficult or misleading clinically, and for this reason the following questions have been raised:

A. *For how long before the Administration of the Urea should Fluids be withheld?*

I believe it is the custom of MacLean and his co-workers to stop all fluids for 6–12 hours before the test, but I would suggest that the necessity for this precaution has not been given sufficient emphasis in all their publications. For instance, Walker (1921), investigating urea concentration tests in the psychoses, allowed tea 2½ hours before the dose of urea. Yet he states that he carefully followed the prescribed details (MacLean and Russell, 1920). It is clear that discrepancies may arise if the test is not carried out under standard conditions. Most healthy active individuals would seem able to concentrate to well over 2 per cent. in the second hour in spite of taking fluids within half an hour to three hours before the urea. Thus H— imbibed 2¼ pints of fluid 1½ hours before the test, and yet his urea percentage was 2·30 at the end of the second hour (Table I, No. 107). On the other hand, patients with

* On behalf of the Medical Research Council.

renal disease may yield very varying concentrations if allowed different quantities of fluid at short intervals before the test.

TABLE I.—*The Effect of taking Fluid shortly before the Test.*

No.	Case.	Quantity of fluid and interval before test.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Remarks.
90	H—	1½ pints; 1 hour	2·80; 62	2·64; 90	3·10; 63	Healthy active individual (*cf.* No. 157).
91	„	1¾ „ 1 „	1·12; 265	2·34; 107	2·85; 82	
107	„	2¼ „ 1½ hours	0·67; 325	2·30; 94	2·29; 86	
113	A—	½ pint; ½ hour	0·59; 230	1·46; 120	2·18; 60	Pyelonephritis.
116	„	¼ „ 3 hours	2·32; 78	2·68; 64	2·36; 49	
132	W—	1 „ 2 „	0·96; 240	1·63; 120	1·52; 120	Chronic nephritis (mixed).
156	„	*Nil* 12 „	2·67; 90	3·03; 45		
33	G. H—	1 pint; 1 hour	0·93; 530	1·45; 130		"Debility." No evidence of renal disease.
42	„	*Nil* 12 hours	2·16; 50	3·69; 50		
157	T—	½ pint; 3½ „	0·91; 330	2·60; 60		History of trench nephritis (*cf.* No. 107).
177	S—	1 „ ½ hour	1·49; 360	1·82; 210		Enlarged prostate.
178	„	*Nil* 12 hours	2·08; 120	2·04; 90		
222	McL—	1 pint; 2 „	2·02; 240	2·20; 240	1·83; 60	Congenital syphilis.
225	„	*Nil* 12 „	2·52; 105	2·50; 90	2·56; 90	
46	N—	1½ pints; 1 hour	0·72; 750	1·65; 120		"Lumbago."
60	„	1 pint; 2 hours	0·96; 235	1·62; 125		
191	„	*Nil* 12 „	2·13; 90	2·46; 66		

I assume that it is desirable to stimulate the kidneys to concentrate to their utmost under the conditions of the test. Two rules should therefore be observed:

(1) Withhold all fluids for at least 6 hours before the dose of urea.

(2) Measure separately the volume of urine passed in each hour subsequently. If the concentrations are well above 2 per cent. the volumes do not matter, but if in the first hour more than 150 c.c. of urine are excreted, or in the second or subsequent hours more than 100 c.c., with a urea concentration below 2 per cent., I would suggest that diuresis may be the cause of the low results and would repeat the test, controlling it by other methods, *e. g.* blood-urea estimation. Even though all fluids are withheld for 6–12 hours beforehand, a few patients may yield artificially low results if they have been taking large quantities of fluid: presumably water has been retained and the urea has enabled the kidneys to eliminate some of it (Table II, Case C—). In diabetes mellitus such polyuria may be troublesome (Table II, Case C. J—). In a few patients with glycosuria I have obtained normal results after their urine has become free from sugar, whereas before treatment the concentration was below 2 per cent. (Table II, Case M—). In the majority of the cases of diabetes, however, the urea concentration has been normal before treatment, often in spite of polyuria (Table II). The most obstinate example of diuresis with which I have met occurred in a patient with diabetes insipidus (Table II, Case D—). Two experiments yielded extremely low results, yet the blood urea

was only 34 mgrm. per 100 c.c. After 1 c.c. pituitrin subcutaneously the concentration at the end of the second hour was 2·94 per cent.

TABLE II.—*Diuresis not Controlled by Withholding Fluids* 8 *to* 12 *Hours before Test.*

No.	Case.	Diagnosis.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Remarks.
379	C—	Unilateral malignant disease of kidney	2·66; 45	2·41; 75	2·36; 60	On ordinary amount of fluids beforehand.
416	„		1·68; 480	1·94; 120	2·00; 120	On large quantities of fluid for several days beforehand.
255	C. J—	Diabetes mellitus	1·59; 230	1·84; 188	2·34; 122	Marked glycosuria.
140	M—	Cirrhosis of liver and glycosuria	1·36; 176	1·87; 130		Marked glycosuria.
267	„			2·32; 178		1st and 2nd hours combined. No glycosuria.
265	R—	Diabetes mellitus	2·32; 150	2·45; 120	2·46; 120	Considerable glycosuria.
275	„		1·84; 90	2·47; 60	2·59; 90	Test performed after treatment. No glycosuria.
51	D—		0·74; 235	0·66; 330		
65	„	Diabetes insipidus	1·15; 142	0·79; 250		Blood urea, 34 mgrm. per 100 c.c.
114	„		2·41; 120	2·94; 120		After 1 c.c. pituitrin subcutaneously.
426	K—	Diabetes mellitus	0·93; 270	2·20; 90	2·26; 60	Test performed after treatment. No glycosuria.
131	H—	Diabetes mellitus	1·35; 166	2·14; 149	2·60; 62	Very slight glycosuria at time of test.

B. *Is* 15 *grm. a Sufficient Dose of Urea in all Cases?*

So much urea may be excreted in the first hour or in the first 2 hours (*e. g.* Table I, No. 222) that there is no longer a sufficient "head" to provoke a concentration which is the maximum for the individual. This may happen even when fluids have been withheld for 8 hours or more (Table II, Case C). Of course where no care has been taken to control diuresis, the resultant polyuria is usually the cause of low results (Table I, Case N).

Secondly, it is conceivable that occasionally the rate of absorption of urea from the alimentary tract may be so delayed that the kidneys have not the opportunity to yield their maximum concentration. Seven cases of gastro-intestinal disease have, however, given normal results (Table III). One case of diabetes and one of chronic parenchymatous nephritis show quite definitely the effect of increasing the dose of urea (Table IV), but it would not be fair in the absence of blood-urea estimations to offer these as evidence of poor absorption. I have not made any experiments with urea given intravenously. The absorption of urea appears to be increased slightly by taking food (*e. g.* breakfast—no fluids) just before the test (Table V).

TABLE III.—*Effect of Gastric and Intestinal Diseases.*

No.	Diagnosis.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	Remarks.
53	Chronic gastritis	2·45; 120	2·53; 90	
101	Appendicitis	4·52; 60	4·65; 90	Mild attack.
169	Carcinoma of stomach	2·09; 90	2·18; 75	Confirmed at autopsy.
195	Carcinoma of transverse colon	3·06; 30	3·09; 60	Confirmed at laparotomy.
312	Duodenal ulcer	1·52; 180	2·75; 85	Confirmed at operation.
57	Cirrhosis of liver and gastritis	3·54; 15	4·02; 45	
62	Cirrhosis of liver and gastritis	2·65; ?	3·20; ?	

TABLE IV.—*Effect of Increasing the Dose of Urea.*

No.	Diagnosis.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	4th hour.	Remarks.
167	Chronic parenchym. nephritis	1·40; 25	1·52; 35			10 grm. of urea. Age 8.
172		1·96; 30	2·12; 30			15 „ „
385	Diabetes mellitus	1·05; 210	1·54; 60	1·68; 90		15 „ „ Age 30.
390		0·83; 180	1·29; 98	1·57; 90		15 „ „
396		1·31; 240	1·98; 120	2·38; 120	2·11; 120	30 „ „ There was no evidence, clinical or otherwise, of renal disease in this patient.

TABLE V.—*Effect of Food (no Fluids) on the Absorption of Urea.*

No.	Diagnosis.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Remarks.
426	Diabetes mellitus	0·93; 270	2·20; 90	2·26; 60	No fluid and no food for 12 hours.
427		1·05; 120	2·03; 120	2·43; 30	No fluid for 12 hours but breakfast one hour before test.
454	Bilateral congenital cystic disease of kidneys	1·53; 30	1·46; 60	1·45; 90	No fluid and no food for 8 hours.
459		2·16; 60	2·24; 30	2·06; 90	No fluid for 8 hours but breakfast (bread and butter) 2 hours before test.
460	Tuberculosis of kidney (unilateral)	1·25; 60	1·53; 60	1·70; 60	No fluid and no food for 8 hours.
461		1·79; 96	1·91; 88	2·05; 64	No fluid for 8 hours but breakfast (bread and butter and 1 egg) 2 hours before test.

c. *To what Extent will Proteinuria Introduce Errors?*

It has long been known that sodium hypobromite reacts with "albumen," liberating nitrogen. On inquiring I found that MacLean and de Wesselow allowed for this by including a certain number of albuminous urines when working out their factor for converting the volume of nitrogen evolved into urea per cent. In the vast majority of instances, from a clinical standpoint, I find that proteinuria makes no appreciable difference. The interpretation of results in the region of 2 per cent., however, may be troublesome. Very occasionally the error is considerable (Table VI). In practice, except when the result is close to 2 per cent., I have found it unnecessary to remove protein unless it exceeds 0·4 per cent. (Aufrecht). Sometimes concentrations, even

TABLE VI.—*Error Introduced by Proteinuria.*

Protein per cent. (Aufrecht).	Influence of removal of protein on urea per cent.			Remarks.	Disease.	No.
	Before removal.	After removal.	Difference.			
0·03	2·05	1·84	0·21	Removal of protein brings result below 2 per cent.	Renal calculus	12
0·09	2·05	2·05	0·00	Protein makes no difference	Trench nephritis	5
0·14	2·33	2·32	0·01	Protein makes no difference	Cirrhosis of liver	267
0·18	2·05	1·87	0·18	Removal of protein brings result below 2 per cent.	Chronic interstitial nephritis	436
0·24	2·61	2·39	0·22	Protein makes little difference to interpretation	Subacute nephritis	133
0·21	2·31	2·26	0·05	Protein makes no difference	Malignant growth of bladder	440
0·35	2·84	2·02	0·82	Protein introduces large error	Malarial nephritis	6
0·39	2·21	1·96	0·25	Removal of protein brings result below 2 per cent.	Albuminuria of pregnancy	442
0·42	1·90	1·66	0·24	Protein makes appreciable difference	Chronic parenchymatous nephritis	31
0·51	0·69	0·63	0·06	Relatively large amount of protein does not matter	Chronic nephritis (mixed)	142
0·66	1·25	1·20	0·05	Large amount of protein makes no difference	Chronic nephritis (mixed)	450
0·75	2·20	1·52	0·68	Proteinuria introduces grave error	Chronic nephritis (mixed)	9

Method of removal of protein.—To 40 c.c. of urine 10 c.c. of 25 per cent. salicyl-sulphonic acid are added. After mixing and filtration, a small portion of the filtrate is tested by further addition of the reagent, to confirm freedom from protein. The urea is estimated in 4 c.c. of the filtrate in the usual way, and the volume of nitrogen evolved multiplied by 5/4. Or, more simply, 5 c.c. of filtrate, which is equivalent to 4 c.c. of untreated urine, may be utilised, the calculation being then as for 4 c.c. of urine.

exceeding this figure, introduce very little error; the urinary proteins of different patients would seem to vary in the readiness with which they react with hypobromite to yield nitrogen. It is interesting to note that urines deeply reddened by blood may not contain as much as 0·4 per cent. (Aufrecht) protein. One of the great advantages of the urea concentration test is that ordinary degrees of hæmaturia do not matter.

ESTIMATION OF CONCENTRATION OF UREA IN BLOOD.

Since urea is very largely exogenous in origin, its concentration in the blood should vary with the nature of the diet. Such has been found to be the case by many observers (MacLean, 1921), and is shown very markedly by estimating the blood urea with and without a dose of urea by mouth (Table IX).

Quite frequently patients with kidney disease are allowed little or no protein. On such diets the blood urea result obtained before an operation (*e. g.* prostatectomy) may sometimes be dangerously reassuring. Furthermore, estimations made on different occasions upon the same patient may not be comparable owing to differences in the diet.

UREA CONCENTRATION FACTOR (U.C.F.).

Unfortunately it is not possible to work out blood-urea curves on similar lines to blood-sugar curves because a satisfactory microchemical method for the estimations is not available. But the conditions would be more standardised if the measurements were made at a known time after a known dose of urea (by mouth). For this purpose it has been my custom for several months to combine the urea concentration test (urine) and the blood-urea estimation. In the majority of cases, after a dose of 15 grm. (by mouth) the percentage in the urine reaches a maximum at the end of the second hour, and stays at about the same level during the third hour. Therefore I have selected the middle of the third hour for estimating the blood urea, and have termed the quotient,

$$\frac{\text{mgrm. urea per 100 c.c. urine passed during the third hour}}{\text{mgrm. urea per 100 c.c. blood in middle of third hour,}}$$

the "urea concentrating power." But MacLean (1921) has already published the principle of dividing the urinary urea by the blood urea, although, to my knowledge, he has not definitely advocated the simple expedient of combining the two tests, and thus simultaneously checking the urea concentration (urine) and standardising the blood urea. I shall therefore adopt the name he suggests, and refer to the combined test as the "urea concentration factor," or, for short, U.C.F. There is always a possible danger in confining investigations to one side of the kidney. The U.C.F. avoids this. The following example illustrates the point well (Table VII).

The patient suffered from chronic nephritis with both chloride and urea retention. His urea concentration (urine) during the third hour after 15 grm. of urea was 1·14 per cent. (volume of urine 90 c.c.) on April 29th, 1921. A month later it was 1·50 per cent. (180 c.c.), and a week after that 1·72 per cent. (60 c.c.). In short, judging by these results alone, the condition of his kidneys would seem to have improved. But the corresponding blood-urea results in

the middle of the third hour were 62, 180 and 240 mgrm. per 100 c.c. respectively, the resulting urea concentration factors being 18, 8 and 7, thus clearly suggesting that the renal disease was worse. Furthermore, a month after the third test the urea concentration had fallen to 1·37 per cent. (90 c.c.), and after a further three and a half months it was 1·33 per cent. (54 c.c.), but contrary to what might have been expected this probably implied an improvement, because the blood urea had fallen to 136 mgrm. and 113 mgrm. (middle of third hour) respectively, with resultant U.C.F.'s of 10 and 12.

TABLE VII.—*Case Illustrating the Importance of Combined Tests (U.C.F.).*

No.	Date.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Blood urea (mgrm. per 100 c.c.). Middle of third hour.	U.C.F.	Remarks.
333	29/4/21	1·24; 50	1·18; 70	1·14; 90	62	18	Urea concentration (urine) rising, but blood urea also rising; U.C.F. falls; kidney lesion probably becoming worse.
351	27/5/21			1·50; 180	180	8	
357	3/6/21	1·83; 120	1·79; 120	1·72; 60	240	7	
363	29/6/21	1·28; 90	1·36; 90	1·37; 90	136	10	Urea concentration (urine) falling, but blood urea also falling; U.C.F. rises; kidney lesion probably improving.
450	13/10/21	1·20; 75	1·28; 70	1·33; 54	113	12	

(For further examples see Table IX, *e. g.* Nos. 461 and 462.)

The obvious criticism of the U.C.F. as thus defined is (1) that the urinary urea is estimated by the hypobromite method instead of by the more accurate urease method, and (2) that the selection of the middle of the third hour for estimating the blood urea is arbitrary. The answer is that the test is suggested as being of greater clinical value than either the urea concentration (urine) alone, or the blood urea without control of diet, or the results of the two tests performed separately; it obviously must yield only an approximation, but in the present state of knowledge of the rate of absorption of urea in different pathological conditions it probably yields a result sufficiently accurate for clinical interpretation; it would only increase the time required for the test to estimate the urinary urea by the urease method, and so in practice would limit its application. Whenever the blood-urea has to be estimated it gives very little extra trouble to arrange to take the sample in the middle of the third hour.

THE UREA COEFFICIENT (AMBARD).

This coefficient has been shown by MacLean and de Wesselow (1920, 1921) to give no more information of clinical value than an estimation of blood-urea.

DISCUSSION OF RESULTS.

MacLean and de Wesselow regard the urea concentration (urine) as normal if between 2 and 4 per cent. at the end of the second hour. Moreover, they say, ". . . if below 2, the condition is unsatisfactory, and the lower the concentration the more serious the lesion" (1920). An example has already been given (Table VII, Nos. 357, 363 and 450) showing that the second half of the sentence quoted is not always strictly true, for occasionally the urea concentration (urine) may fall because the blood-urea has also fallen, the lesion presumably having improved. At the present time I would interpret the results of this test as follows: If the urea concentration (urine) at the end of the first, second or third hour exceeds 2·5 per cent. it is "normal," *i. e.* probably more than one-quarter of the total kidney tissue was functioning on the day of the test; probably little or no further information of value clinically would be obtained by estimating the blood urea. If the concentration lies between 2 and 2·5 per cent. it is probably "normal," but it would be safer to check it by estimating the blood urea (preferably 2½ hours after the dose of urea). If the urea concentration is below 2 per cent. it may be due to an artefact, or the renal condition may be definitely unsatisfactory, less than one-quarter of the kidney functioning. As examples of artefacts there may have been diuresis, the full dose may not have been taken, or the dose may even have been omitted, the solution may not have contained 15 *grammes* (sometimes 15 *grains* have been prescribed in error!), and so on; if there is any reason to suppose that the test has not been carried out correctly it should be repeated. An estimation of urea in the 24 hours' urine may serve as a useful check; if it is higher than the second- or third-hour samples the test has not been

TABLE VIII.—*Urea in Urine above* 2 *per cent., and Blood Urea above* 40 *mgrm.*

No.	Disease.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Blood urea. (No urea by mouth.) (Mgrm.)	Remarks.
153	Subacute nephritis	1·61; 70	2·08; 90	2·24; 70	49	Recovery.
388	Vesical calculus	2·16; 90	2·32; 90	2·41; 60	73	Successfully removed by operation.
405	Enlarged prostate	1·84; 30	2·26; 30	2·13; 15	44	Successful prostatectomy.
379	Malignant tumour of kidney (unilateral)	2·66; 45	2·41; 75	2·36; 60	92	Successful nephrectomy.
436	Chronic interstitial nephritis	1·87; 90	2·06; 60	1·90; 60	85	Blood-pressure(systolic) 260. Marked albuminuric retinitis.
447	Chronic nephritis	1·96; 90	2·00; 120	1·99; 60	76	Marked dyspnœa. Blood-pressure 190/120. Fundi normal. Wassermann reaction positive.

TABLE IX.—*Results Obtained by Combined*

GROUP 1.—Urea Concentration

No.	Disease.	Date	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.
370	Talipes equino-varus	8/7/21		3·80; 90	4·73; 60
378	Chronic nephritis (mixed)	12/7/21	2·98; 36	3·16; 32	
400	Calculus in right kidney; tuberculosis of left kidney	29/7/21	2·03; 30	2·42; 60	2·19; 30
432	Diabetes mellitus	19/9/21	3·09; 100	3·11; 90	3·10; 85
360	Liporhabdomyoma of left kidney	6/6/21	4·00; 120	4·04; 60	3·99; 60
367	L. pyonephrosis	4/7/21	2·11; 120	2·08; 30	2·09; 30
453	Postural proteinuria (? functional)	15/10/21	2·33; 70	2·48; 70	2·70; 70
426	Diabetes mellitus	13/9/21	0·93; 270	2·20; 90	2·26; 60
430	Diabetes mellitus	16/9/21	2·42; 120	2·64; 120	2·57; 90
372	Malignant growth of bladder	11/7/21	2·48; 45	2·81; 60	2·88; 90
405	Enlarged prostate	30/7/21	1·84; 30	2·26; 30	2·13; 15
455	Carcinoma of stomach and chronic nephritis (interstitial)	15/10/21	2·03; 75		2·18; 86
436	Chronic nephritis (interstitial)	26/9/21	1·87; 90	2·06; 60	1·90; 60
388	Vesical calculus	22/7/21	2·16; 90	2·32; 90	2·41; 60
379	Malignant tumour of kidney (unilateral)	12/7/21	2·66; 45	2·41; 75	2·36; 60
447	Chronic nephritis	8/10/21	1·96; 90	2·00; 120	1·99; 60
	GROUP 2.—Urea Concentration				
399	Diabetes mellitus	29/7/21			1·41; 180
337	Post-scarlatinal nephritis	5/5/21	1·43; 60	1·62; 90	1·38; 60
393	Enlarged prostate	25/7/21	1·77; 30	1·47; 60	1·83; 90
404	Enlarged prostate	30/7/21	1·55; 30	0·93; 5+	1·32; 15+
374	Enlarged prostate	11/7/21	1·04; 60	1·24; 90	1·75; 30
330	Chronic interstitial nephritis	26/4/21	0·95; 90	1·17; 120	1·04; 60
444	Chronic interstitial nephritis	30/9/21	1·38; 120	1·26; 120	1·39; 120
366	Malignant prostate disease	2/7/21	0·55; 68	0·59; 30	0·69; 30
307	Chronic interstitial nephritis	31/3/21	0·71; 90	0·78; 60	0·83; 60
371	Right renal calculus; left cystic kidney	11/7/21	1·49; 60	1·02; 60	1·16; 90
448	Perinæal fistula	12/10/21	1·03; 45	1·21; 60	1·38; 45
	GROUP 3.—Example of identical U.C.F. for two cases,				
461	Tuberculosis of left kidney	21/10/21	1·79; 96	1·91; 88	2·05; 64
462	Renal calculus	21/10/21	1·59; 68	1·68; 93	1·70; 74

Remarks on Nos. 461 and 462.—Judging by urea concentration test (urine) alone, 461 would seem
judging by the U.C.F. the two

Tests (Urea Concentration Factor).

(urine) 2 per cent. or higher.

Blood urea 2½ hours after dose of urea. (Mgrm.)	U.C.F.	Date.	Blood urea. (No urea by mouth.) (Mgrm.)	Remarks.
66	72	9/7/21	27	No evidence whatever of renal disease. Successful cuneiform osteotomy of foot.
53*	60	...	...	*Blood urea two hours after dose of urea.
37	59	...	...	Successful right nephrolithotomy.
58	53	...	...	No evidence whatever of renal disease.
75	53	...	...	Successful nephrectomy.
42	50	...	...	Successful nephrotomy.
55	49	...	...	Wassermann reaction, weak positive.
53	43	...	...	No evidence whatever of renal disease.
63	41	...	...	No evidence whatever of renal disease.
70	41	13/7/21	34	Growth partially excised.
59	36	2/8/21	44	Successful prostatectomy.
78	28	...	...	Inoperable carcinoma.
92	21	23/9/21	85	Blood-pressure 260. Marked albuminuric retinitis.
131	18	25/7/21	73	Successfully recovered by operation.
133	18	14/7/21	92	Successful nephrectomy.
134	15	14/10/21	76	Marked dyspnœa. Blood-pressure 190/120. Fundi normal. Wassermann reaction positive.
(urine) below 2 per cent.				
34	41	...	...	Pyuria. Pyæmia. Death from septic pneumonia. Low urea in urine may be due to artefact.
45	31	...	...	Slight hæmaturia.
91	20	...	...	Successful prostatectomy.
73	18	...	...	Survived suprapubic cystotomy. Died from uræmia within thirty-six hours of prostatectomy.
104	17	...	...	Successful subrapubic cystotomy. Died twelve days after prostatectomy from exhaustion. Clinically not uræmic.
66	16	...	...	Systolic blood-pressure 190. Albuminuric retinitis.
97	14	...	...	Systolic blood-pressure 160. Fundi normal.
85	8	...	...	Inoperable. Discharged from hospital.
105	8	19/3/21	67	Systolic blood-pressure 240.
140	8	...	...	General condition good. No clinical evidence of uræmia. Operation considered inadvisable.
165	8	...	...	Uræmic at time of test. Died few days later. Fractured pelvis 2/9/21. Ruptured urethra.
(1) belonging to Group 1, (2) belonging to Group 2.				
59 48	35 35	...		Same patient as No. 400, three months later.

to be the less severe; judging by the blood urea alone, 461 would appear the more serious; cases are about equally severe.

properly conducted. In the absence of any artefact, results below 2 per cent. should always be controlled by estimating the blood-urea (preferably 2½ hours after the dose), for they may be accompanied, for example, by a practically normal blood urea (Table IX, No. 337) or by a very high one (Table VII, No. 357), the first being a much less severe case than the second.

The normal figures for blood-urea on ordinary hospital diets are usually given as 15–40 mgrm. per 100 c.c. (MacLean, 1921), and it is generally considered that if the urea concentration (urine) is above 2 per cent. the blood-urea will be below 40 mgrm. But I have encountered a few cases with urea in urine above 2 per cent. and blood-urea above 40 mgrm. (Table VIII).

Walker (1921) has reported similar findings. Some writers have suggested that factors other than the diet or the kidneys may influence the level of the blood urea—for example, the toxæmia of acute febrile diseases (Wagner, 1920) or of acute intestinal obstruction (Louria, 1921). If this be so, it is still more important to estimate simultaneously the urea concentration in both blood and urine in assessing renal efficiency.

I have not examined a sufficient number of active healthy individuals to suggest what may be the "normal" blood-urea level 2½ hours after a dose of 15 grm. by mouth. In 40 hospital patients, however, with a urea concentration (urine) over 2 per cent., the lowest has been 37 mgrm. and the highest 133 mgrm. per 100 c.c. under these conditions (Table IX). Again, owing to the small numbers of observations, I cannot give "normal" figures for the "urea concentration factor"; but so far, in 40 patients with a urea concentration (urine) over 2 per cent., the highest has been 72 and the lowest 18 (Table IX). These results seem to support the hypothesis that urea is a "no-threshold" substance.

As has been emphasised elsewhere (Harrison, 1921), in estimating renal efficiency it is necessary to weigh up all the evidence, both clinical and laboratory. After studying U.C.F.'s it is not surprising that it has been found clinically that the urea results are not absolute. For example, W. S— died from uræmia within 48 hours of cystoscopy; his urea concentration (urine) was 1·29 per cent. (urine 120 c.c. second and third hours combined) three days before the examination. On the other hand, R— is still alive several months after decapsulation of both kidneys, with a urea concentration shortly before the second operation of 1·18 per cent. (urine 70 c.c.). Again it is probably unsound to state that " . . . In no case should an operation necessitating a general anæsthetic be undertaken on any patient whose blood contains 100 mgrm. or more of urea" (MacLean, 1921). In some cases the conditions under which the estimation is made may profoundly affect the result; and the other findings (clinical and laboratory) may not contra-indicate operation in a few instances, in spite of high blood-urea. Thus McK— had a blood-urea of 100 mgrm. (low protein diet, no urea by mouth), and yet suprapubic cystotomy for malignant prostatic disease (under a general anæsthetic) was quite successful, the patient being discharged from hospital with a permanent drain, whereas another patient, L—, with a blood urea of 37 mgrm. (full hospital diet), died from uræmia three days after prostatectomy. "Rules," such as the one quoted above, may be fully justified in the majority of cases, but a small minority of patients may suffer as a result of their too rigid application,

TABLE X.—*Examples of Nephritis with Œdema Treated with Urea.*

No.	Urea retention.	Urea per cent. and c.c. of urine at end of— 1st hour.	2nd hour.	3rd hour.	Blood urea (mgrm. per 100 c.c.) (No urea by mouth.)	Œdema before treatment.	Dose of urea. (Grammes.)	Duration of urea treatment.	Results and remarks.
268	Not tested .	...	3·16; 90	...	...	+++ Could scarcely open eyes	16 daily	2 weeks	Œdema almost completely disappeared in forty-eight hours.
133	...	2·13; 105	2·39; 60	...	...	+++	15 b.d.	4 „	Œdema disappeared in three weeks.
166	Present . .	1·41; 120	1·51; 90	...	59	+++	15 „	2 „	Same case as 133 with return of œdema after two months at home. Œdema disappeared in one week.
138	Slight . .	0·73; 60	1·02; 110	1·40; 16	45	+++	15 „	8 „	Œdema much reduced by urea, and completely disappeared after a further two months on full protein diet.
221	Considerable .	1·49; 60	1·52; 60	1·70; 60	88	++++	15 „	3 „	Urea no effect. Perhaps dose not large enough. Œdema slightly increased in spite of the urea.

and occasionally it may be forgotten that the converse does not necessarily hold.

Value of urea tests in prognosis.—Though the tests are not "absolute," repeated estimations at intervals, under standard conditions, on any given patient may be most helpful in following the progress of the disease (Table VII), although it is fair to question whether small variations have any clinical meaning. Obviously urea tests are but one link in the chain of evidence upon which a prognosis should be based.

Value of urea tests in treatment.—Whenever urea is used in the treatment of œdema in nephritis (MacLean and Russell, 1920; MacLean and de Wesselow, 1919) it would seem useful to perform urea tests beforehand. When chloride retention is unaccompanied by urea retention the treatment may be expected to work well. In one case (Table X, No. 268) urea worked like a charm, the œdema almost completely disappearing in 48 hours. But usually, as pointed out by the same authors, it is necessary to continue the urea (15 grm. twice daily) over a period of weeks or months. Frequently, in my experience, chloride retention is accompanied by urea retention. In such cases it would be expected either that urea would be of no avail, or that very large doses would be necessary to reduce œdema. In two instances when there was urea retention, œdema completely disappeared after a few weeks of urea together with the usual treatment for nephritis. In one patient the œdema slightly but steadily increased in spite of 30 grm. of urea daily for three weeks (Table X).

Occasionally patients complain that the repeated doses of urea cause nausea and headache, and rarely, vomiting follows. I find that autoclaving the mixture of urea 15 grm., tincture of orange 1 c.c. and water to 100 c.c. makes the taste slightly less unpleasant, and also stops the growth of a fungus which otherwise may occur if the solution is kept for a few days.

SUMMARY.

When applying the provocative urea concentration test:

(*a*) It is advisable to withhold fluids for at least six hours before the test.

(*b*) It is safest to measure the volume of urine passed each hour, even though the urea be not estimated in all samples. A volume greater than 150 c.c. (first hour), or 100 c.c. (second or subsequent hours), generally signifies excessive diuresis when a result below 2 per cent. does not necessarily imply an inefficient kidney. The test should be repeated and controlled by estimating the blood urea.

(*c*) Occasionally it may be impossible to bring diuresis within these limits. Other tests are then essential. In one case of diabetes insipidus diuresis was controlled by 1 c.c. of pituitrin subcutaneously.

(*d*) 15 grm. may not always be sufficient to provoke a concentration greater than 2 per cent., although the kidneys be efficient.

(*e*) Proteinuria exceeding 0·4 per cent. (Aufrecht) may introduce a large error.

Blood-urea estimations may be misleading (*e.g.* before operation) if the nature of the diet is not considered.

By simply combining the urea-concentration test (15 grm. by mouth) and

the blood-urea estimation, the former is checked and the latter is standardised. The "urea concentration factor" (U.C.F.) so obtained is defined thus:

$$\frac{\text{mgrm. urea per 100 c.c. urine passed during third hour}}{\text{mgrm. urea per 100 c.c. blood obtained in middle of third hour.}}$$

Determinations of U.C.F. indicate that sometimes a "normal," or almost "normal" urea concentration (urine) may be due to a high blood urea, and illustrate the importance of estimating simultaneously the urea concentration in blood and urine.

The "normal" values for the urea concentration test (urine), the blood urea and the urea concentration factor are discussed.

The use of the urea tests in the prognosis of renal disease and in the treatment of œdema in nephritis is briefly mentioned.

I wish to thank the members of the staff at King's College Hospital for the opportunities of performing the above tests on their patients.

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EXPERIENCES WITH THE SCHICK TEST AND ACTIVE IMMUNISATION AGAINST DIPHTHERIA.

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AFTER a preliminary investigation into the value of the Schick test and active immunisation against diphtheria (Copeman, 1921), the Ministry of Health instituted a more extensive test at the Mitcham Poor-Law Schools with the consent of the Guardians. In the present paper the immunological results are described.

The Mitcham schools contain a resident population of rather over 300 children between the ages of three and sixteen years. All, until quite recently, not only lived, but received their education, in one or other of the three adjoining institutions. The weekly rate of admission and discharge is small, and the population is thus a very stable one. Prior to November, 1920, these schools had been practically free from infectious disease, but since then they have been affected with scarlet fever and diphtheria almost continuously. No case of diphtheria has, however, been notified since August 4th, 1921.

The general scheme of work included a routine swabbing of all children and testing the bacilli isolated for virulence. The Schick test was applied in every case and the positives immunised with toxin-antitoxin mixtures, the effect of this being judged by re-testing by Schick's method at a later date.

BACTERIOLOGICAL EXAMINATION.*

All the children in the school and annexed infirmary, numbering 329, were swabbed on two separate occasions. The first set of swabs, hereinafter referred to as Series I, was taken eight weeks before the second set, called Series II. Children found on either occasion to be harbouring bacilli morphologically resembling *B. diphtheriæ* were re-swabbed until three consecutive negatives were obtained.

* The whole of the technical work was planned and carried out by the staff of the Wellcome Physiological Research Laboratories, the bacteriology being done by one of us (A. J. E.), Dr. Okell and Miss Baxter, the examination of the blood samples for antitoxin and the control and testing of the Schick toxin and toxin-antitoxin mixtures by A. T. G. and Miss Allen, the Schick testing and the inoculations with toxin-antitoxin mixtures by R. A. O'B.

In all some 1400 swabs were examined by cultural methods. The following results were obtained:

No. of Cases Diagnosed as "M.D." in Series I.

"M.D." = bacilli morphologically resembling *B. diphtheriæ* in smear from "Loeffler" overnight culture.

"M.D." throat only	1
"M.D." nose only	8
"M.D." nose and throat	9
Total positive	18
Total swabbed	329
Percentage positive	5·47

Cultures of B. diphtheriæ *Isolated in Series I.*

	"M.D." diagnosis.	Cultures isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Throat	1	1	1	0	1	0
Nose	8	3	1	2	2	1
Throat and nose	9	4*	3	1	2	2

"Correct" sugar reactions were acid production in glucose, but not in saccharose. Virulence was tested by the intracutaneous method checked by the subcutaneous (Eagleton and Baxter, 1921).

* Four cultures from two patients who both harboured virulent and avirulent strains, but at different times.

No. of Cases Diagnosed as "M.D." in Series II.

"M.D." throat only	1
"M.D." nose only	3
"M.D." nose and throat	2
Total positive	6
Total swabbed	327
Percentage positive	1·8

Cultures of B. diphtheriæ *Isolated in Series II.*

	"M.D." diagnosis.	Cultures. isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Throat	1	0	0	0	0	0
Nose	3	2	2	0	0	2
Nose and throat	2	2	2	0	0	2

Results of Series I and II Compared.

	"M.D." diagnosis.	Cultures isolated.	Sugar reactions. Correct.	Not done.	Virulent.	Avirulent.
Series I	18	8	5	3	5	3
Series II	6	4	4	0	0	4
Common to both	3	2	2	0	0	2
Total	21	12	9	3	5	7

The swabs in Series I were taken at the end of a series of cases of clinical diphtheria, when opportunities for infection of throats were fairly plentiful; one would naturally expect that the later series, taken at a date two months further away from the existence of manifold opportunities for infection, would show a lower reading of infection, and this is what we found.

With regard to the difficulties of isolation, one or two short notes may be made. The digestion of Loeffler's medium by organisms occurring in the throat and nose, but especially the latter, is one fruitful source of trouble. Another great difficulty consists in the fact that "M.D." may be present in very small numbers indeed. We do not think it is going too far to say that the ease with which *B. diphtheriæ* can be isolated from the nose or throat is a measure of the abundance of this organism in the nose or throat, and therefore, most probably, of the danger to the community arising from the patient under examination.

In our opinion it follows that the percentage of cases from which virulent *B. diphtheriæ* can be isolated is more important than a carrier rate based on morphological criteria, which disregard the pathogenic power of the organisms found and the number present.

In support of this view, and as a contrast to the results in these two series, the following brief account of some work that has been largely contemporaneous, and so has acted as a control, will be of interest. One hundred and fifty convalescents from clinical diphtheria were swabbed at different intervals after the disease. In 14 "M.D." was diagnosed, and although one swab only from each case was submitted to us, from 13 out of those 14 virulent *B. diphtheriæ* were isolated.

In Series I five virulent carriers were found, one of them being convalescent from clinical diphtheria at the time of swabbing. The most persistent gave an initial "virulent"; subsequent cultures were "avirulent." In Series II no "virulent" carriers were found.

There had been a series of cases of clinical diphtheria in this institution, and it was to be expected that one or more carriers of virulent bacilli, easily isolated and continuously excreted, would be found. No such carrier was, however, discovered. But our examination was not made at the height of the epidemic, and we know from general experience (*cf.* also Hartley and Martin, 1920) that most convalescents rapidly become clear of bacilli. It may be that one of the five "virulent" carriers (or, less probably, one of the seven "avirulent" carriers) was the cause of the epidemic, no evidence having been obtained that the infection was derived from a source outside the institution.

SCHICK TESTING.

Toxin.—The same toxin was used throughout. Fresh dilutions were made for each day's work, and the potency of the diluted toxin remaining over was tested on guinea-pigs (Glenny, Allen and O'Brien, 1921). We adopted the original Schick formula as used by Park and Zingher, 0·2 c.c. containing $\frac{1}{50}$ guinea-pig M.L.D. being injected. In one group of about thirty children in which the first Schick test had given rise to some slight uncertainty, it was repeated, the original Park formula being used simultaneously with Zingher's later modification, in which 25 per cent. more toxin is used on the left arm

and 50 per cent. extra toxin in the heated control on the right arm. In this small group of cases we could not find that the Zingher modification gave any clearer readings than the original formula.

Readings.—In a discussion of the reaction it is necessary to have concise descriptive signs or terms which should convey as much information as possible. We think this question is so important that we append a short table of explanation of the conventions that we would suggest.

Schick Test Nomenclature.

Type of reaction.	Written description of reaction.	Verbal description of reaction.	Description of patient.	Description previously in use.
1	−	Negative	Immune = I.M.	Negative.
2	− (ψ)	Negative and pseudo	Immune (pseudo reactor) = I.M.P.	Pseudo.
3	+	Positive	Non-immune = N.I.M.	Positive.
4	+ (ψ)	Positive and pseudo	Non-immune (pseudo reactor) = N.I.M.P.	Combined.

The readings were made daily up to five or seven and occasionally thirteen days. If one decides to make one reading only, the most satisfactory is that made from the fourth to the seventh day after the injection.

The results of our tests are set out in Table I. The percentage of positive reactions obtained for this group of children, between the ages of three and sixteen, correspond closely with that published by Park.

TABLE I.—*Schick Test Results at Various Ages.*

Age in years.	Immune and immune (pseudo reactor).	Non-immune.	Total.	Per cent. Immune and immune (pseudo reactor).	Per cent. Non-immune.
3–4	9	3	12	75·0	25·0
5–6	20	8	28	71·4	28·6
7–8	30	12	42	71·4	28·6
9–10	39	22	61	63·9	36·1
11–12	55	21	76	72·4	27·6
13–14	51	23	74	68·9	31·1
15–16	23	13	36	63·9	36·1
Total	227	102	329	69·0	31·0

Technique and reading of results.—So far as the technique is concerned, we have but little to add to the excellent description in the publications of Park and Zingher. The needle and syringe must work without any defect; the slightest bluntness of the needle or leak of the plunger at the junction of the needle results in unsatisfactory work. We used a Burroughs Wellcome No. 1 dental needle with a 1 c.c. all-glass syringe, and a 1 c.c. long

tuberculin syringe; in one series a "Record" 1 c.c. syringe was used. It is most convenient to use two syringes, one for the toxin, the other for the control; these should be of the same make, with the plunger working equally well, and the needles should be equally sharp, otherwise a slight difference in the depth at which the intradermic injection is made may occur, with resultant blurring of the readings, particularly those of the first day. The control syringe may be identified by a rubber band fixed around the barrel.

Readings were made daily in most of the cases up to five or seven days, each day's reading being made without reference to the previous readings. Wherever there was any discrepancy the Schick test was repeated. Fifty-five of the children were thus re-tested because some slight doubt or discrepancy had occurred in the course of the first test (Table II).

TABLE II.—*"Doubtful" Schick Results among 329 Children Tested.*

	First Schick test. Readings regarded as probably:			Second Schick test.	
	− & − (ψ)	+ & + (ψ)		− & − (ψ)	+ & + (ψ)
	44			44	
		11		4	7
Total	44	11 = 55		48	7 = 55

Consideration of these cases shows that when a reading is doubtful, it proves on further investigation in the great majority of cases to be negative or "negative (and pseudo.") When deciding that a given reaction is "negative (and pseudo)," one has always present in one's mind a slight fear that the reaction may be "positive (and pseudo)." Fortunately these latter reactions are rare. Of the 329 children only two showed a "positive (and pseudo)" reaction, *i. e.* on the right arm a reaction which, although smaller, resembled the left in depth of colour and degree of desquamation.

Table III gives details of the four cases in Table II in which differences were obtained on re-testing.

TABLE III.—*Apparent Discrepancies.*

	First test.	Second test.	Blood sample.
1.	Positive (and pseudo)	Negative (and pseudo)	More than $\frac{1}{30}$ unit of antitoxin per c.c. of blood found in every case.
2.	Positive	Negative (and pseudo)	
3.	Positive	Negative (and pseudo)	
4.	Positive (and pseudo)	Negative (and pseudo)	

On referring to the readings we had recorded in the first test, we found in every case that the final entry, on which the child was classed, recorded a "very faint" or "very, very faint" stain on the left arm, greater in size than on the right, and that some of the readings had suggested a "negative (and pseudo)" reading. With the experience gained in these cases and the help supplied towards the interpretation of doubtful readings by the determination of antitoxin in the patient's blood, we should, with similar readings now, return the case as "immune (pseudo reactor)."

Blood samples.—Our confidence in the ultimate accuracy of our classification of the children as immune or non-immune is founded partly on the results of the repeated Schick tests in "doubtful" or difficult cases, but to a greater extent on the interpretation of the titration of antitoxin in the blood of individual children by the Römer method (Glenny and Allen, 1921). This we regard as a very important part of our investigation.

Many observers state that an antitoxin content per c.c. of blood of less than $\frac{1}{30}$ unit of antitoxin will fail to neutralise the toxin injected in the Schick test and so make the readings positive, while, with a greater antitoxin content, a negative Schick reaction will result. Our own experience in several hundred tests has hitherto afforded no ground for disagreement with this statement.

Sixty-six blood samples were taken and the antitoxin titrated. The findings are shown in the table.

TABLE IV.

Antitoxin per c.c. of blood.	No. of cases.	
5–20 units	7	8 of these children had had toxin-antitoxin mixtures injected.
2–5 ,,	8	
1–2 ,,	15	
$\frac{1}{2}$–1 unit	9	
$\frac{1}{5}$–$\frac{1}{2}$,,	9	
$\frac{1}{100}$–$\frac{1}{10}$,,	5	
? $\frac{1}{1000}$,,	5*	
Less than $\frac{1}{2000}$ unit	8	

Note.—In many instances the sample of blood for titration of antitoxin was not taken until approximately a week after the Schick test was done.

Recent experiments on animals (yet to be published) show that the minute amount of toxin used in the Schick test is sufficient, under certain circumstances, to cause the development of such a degree of immunity that an animal which has just given a non-immune Schick positive result, will, a week or two later, when the test is repeated, give an immune negative response. This may possibly be the explanation of some of the discrepancies recorded.

It is probable that these results will be confirmed on the human subject, but the point will be dealt with in a subsequent paper.

Error on first day's reading.—It is obviously of great importance in the presence of an epidemic of diphtheria to be able, if possible, to make a decision that the given patient is immune or not within the first twenty-four hours of the Schick test. We did in all approximately 400 Schick tests. In four instances the first day's reading was entered as "negative," whereas later readings showed that the reaction was "positive." First day's readings of the reaction in eight children were "positive," but the later readings showed clearly that the reaction was "negative" and the children therefore immune. In nine instances the first day's reading entered as "doubtfully" positive or negative differed from the final reading. Thus an actual error was made in

* All of these five sera, when injected intradermically without toxin into guinea-pigs, gave some reaction; it was, therefore, difficult to estimate the antitoxin content with the small amounts of serum available.

twelve cases and a dubious but erroneous reading in nine, *i.e.* 21 in all. In 400 tests, therefore, a decision based on the first day's reading proved erroneous in 5 per cent. of the cases.

"*Carriers.*"—The following table gives the results of the Schick test and examinations of the blood of children who harboured "morphological diphtheria bacilli." It is to be noted that the carriers of virulent bacilli possessed a fairly high degree of immunity.

TABLE V.

	Virulent cultures isolated.	Avirulent cultures isolated.	"Hofmann" isolated.	"M.D." not isolated.
	5	7	4	6
Schick test positive	0	1	2	1
Schick test negative	5	6	2	5
Blood samples examined	5	1	—	3
Antitoxin content per c.c.	4, 1 unit 1, $\frac{1}{50}$ unit*	< $\frac{1}{2000}$		1. < $\frac{1}{2000}$ 2. $\frac{1}{2}$–1 3. 2–5

Note.—In some of these instances the sample of blood for titration of antitoxin was not taken until approximately a week after the Schick test was done.

ACTIVE IMMUNISATION.

All children giving a positive Schick reaction, 102 in all, were given three weekly doses of 1 c.c. of toxin-antitoxin mixture (Park-Zingher formula and American official standard). As a precautionary measure, in most cases a preliminary injection of 0·05 c.c. had been given.

Reactions.—The results were reassuring. Of the 102 children 17 were reported to the nurse (one child twice). Eight had a temperature of 102°, four of 101°, and two of 99°. Though this number (17) seems large, the reaction was so slight in all but three of the children that they did not wish to stay in bed for a whole day. One of the remaining three wished to stop in bed for two days.

W. G—, one of two that were ill, had a temperature of 102°, and was distinctly ill for three days after her first dose of 0·05 c.c. A week later she received 0·01 c.c., and at intervals of five days 0·05 c.c.; 1 c.c., 1 c.c., 1 c.c. She remained quite well throughout.

It is therefore probable that the initial illness was due to some other cause than the injection of the mixture.

The other child, A. B—, vomited immediately after the first injection, and was ill for two days. Inquiry showed that she had had a pork dinner a few hours before the injection, and had suffered from abdominal pain before the injection. Unfortunately this was not known to us.

The constitutional reactions were slight. Local reactions, on the other hand, were at first rather alarming. In approximately half of the cases

* Patient G. M— referred to under "Results of Immunisation" (p. 49).

a large flushed area varying from 2 in. by 1 in. to even 6 in. by 3 in. appeared, but rapidly cleared up after the first twenty-four to forty-eight hours. We were very disturbed until we learned by experience that a large, angry-looking swelling would not prevent a boy from playing football two days later or a girl from skipping on the first day after the injection, and that it did not interfere to any great extent with sleep.

Results of Immunisation.—Approximately eleven weeks after the conclusion of the course of immunisation the whole of the children in the school were again subjected to the Schick test.

Of the 227 children who had been classified by the first Schick test as immune, 203 remained in the school; of these 201 again gave negative or negative (and pseudo) reactions, while two children showed positive reactions. Samples of blood were obtained from these two, and neither contained any antitoxin. Both children had had diphtheria, and had been removed to hospital, where they were given antitoxin shortly before the first Schick test was done. It appears probable that the first Schick test, which in one child was undoubtedly negative and in the other (G. M—) had been read as "pseudo (with a faint possibility of positive)," had been influenced by some remnants of the antitoxic serum which had been injected in hospital. Before the second Schick test was performed, some three months later, the last remnants of the horse antitoxic serum had been excreted, and the Schick results were therefore positive.

Of the 102 children who had given a positive reaction and had been inoculated with a toxin-antitoxin mixture, 99 remained and were re-tested. Two still gave a clear positive reaction; samples of their blood were tested; no antitoxin was present in either. These children will be re-inoculated.

CONCLUSIONS.

(1) In a residential school of 329 children, amongst whom a recent epidemic of diphtheria had occurred, eighteen (6 per cent.) carried morphological *B. diphtheriæ* in throat or nose. At the first swabbing, at the end of the epidemic, 5 per cent. of carriers were found; at the second, two months later, 2 per cent. From these eighteen children twelve cultures were isolated, of which five were virulent.

(2) The later swabbings from these five carriers showed either avirulent organisms or no "morphological diphtheria" bacilli.

(3) On the grounds of a bacteriological examination of a large number of cases of diphtheria, carriers and convalescents, the following suggestions are tentatively put forward:

(i) That the "avirulent carrier" is of no importance epidemiologically.

(ii) That the danger of a carrier of virulent bacilli is, at the time of examination, proportionate to the number of virulent bacilli present in throat and nose, and, therefore, to the ease with which the virulent bacilli can be isolated.

(4) Of 329 children, aged from three to sixteen, 102, *i. e.* 31 per cent., gave a positive Schick reaction; 95 per cent. of the readings made on the first day proved to be accurate.

Of the 227 children who had given a negative response when first

Schick tested, 203 remained in the school eleven weeks later. Of these, 201 on being re-tested again gave a negative or a negative (and pseudo) response, thus confirming the decision made two months previously. Two showed a positive response at the second test; these two children had had antitoxin injected shortly before the first test was made.

(5) These 102 children were inoculated with toxin-antitoxin mixtures (Park and Zingher formula). Local reactions occurred in about one-third of the children; though in some of these the area of inflammation was large, the activities of the children were but little interfered with. Constitutional reactions were slight in all but two of the children.

(6) Of the 102 children, 99 were still present in the school eleven weeks later when the Schick test was repeated. Two gave an undoubtedly positive response, the remainder a negative or negative (and pseudo) reaction; 98 per cent. were therefore immune.

We have much pleasure in thanking Dr. Manby for interesting the Guardians in the test, and Dr. Morrish, the Medical Officer; Mr. Drury, the Superintendent of the Schools; Mrs. Drury, the Matron, and the Infirmary Sister, for the valuable assistance afforded by them, especially in arranging practical details in connection with the work.

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THE CARBOHYDRATE METABOLISM OF SURVIVING MOUSE TISSUES AND TUMOURS.

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From the Laboratories of the Imperial Cancer Research Fund.

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THE present communication may be regarded as a continuation of a paper by the author and W. H. Woglom (1920), published in the first volume of this journal. On that occasion a method was described of ascertaining the gaseous exchange of surviving tissues of the mouse, and especially of arriving at the ratio of the carbonic acid output to the oxygen intake—that is, the respiratory quotient. The reader is referred to that paper for the details of the procedure adopted, which may here be briefly described as consisting in the measurement of the changes of volume observed in an air space containing a known amount of finely-divided tissue or tumour suitably disposed to facilitate the respiration of the cells. The apparatus employed was a modification of the well-known Barcroft blood-gas analyser, and the same instruments, three in number, have been used for the experiments about to be recorded.

A series of observations done in the above manner gave differences, which we regarded as significant, in the case of the several normal tissues examined, namely kidney, liver, mamma, submaxillary gland and embryo. Six strains of transplanted tumours gave still greater differences in their average respiratory quotient, and the quotient of the more rapidly growing tumours was found to be higher than that of the more slowly growing tumours. Interpretation of these results in terms of the food-stuffs being burnt led to the general conclusion that the rapidly growing neoplasms katabolised more carbohydrate, whilst those of slower growth burnt mainly fat. One notable exception to this generalisation was found with a tumour of medium rate of growth, which, nevertheless, gave the highest quotient found. This particular strain was distinguished in another respect from its fellows, as its cells always contained a considerable amount of glycogen. Consideration of these facts at once raised the further question: Does a low respiratory quotient mean that a tumour strain can only consume carbohydrates to a limited extent, or does it only mean that in the parenchyma there is no readily available supply? A comparison of the results obtained with normal kidney and liver showed that with an abundant supply of glycogen in the case of liver, the quotient found was appreciably lower than that of kidney which was free, at least histologically, from glycogen. Liver, also, did not react in the same way to its carbohydrate content as the glycogen-bearing tumour mentioned above. Further experiments were necessary to test the question, and these took the

line of adding small amounts of different sugars to the tissue emulsion before placing in the respirometer bulb.

The sugars tested were in all cases made up into a solution containing two parts pro mille. As diluent, Ringer solution could not be used because of the bicarbonate present, and a 0·85 per cent. saline solution made with tap water was adopted. The tissue emulsion was spread in a thin uniform layer on one side of a rectangular slip of tissue paper which was then floated on a small pool of sugar solution. The emulsion adheres to the filter-paper, and allows the excess of solution to drain off. It was next placed on a slip of glass, and introduced into the respirometer bulb, where the further treatment was exactly similar to that already described in the previous communication.

The taking up of sugars by surviving tissues such as the mammalian heart has been the subject of investigation by a number of experimenters, and the points of more immediate interest will be found in the papers by Locke and Rosenheim (1904), Rohde (1910), Neukirch and Rona (1912), Maclean and Smedley (1913), Evans (1914), and Starling and Evans (1914). Usually the consumption of glucose has been estimated from analysis at intervals of the perfusing fluid. A useful summary of this work is given by Starling and Evans, who have further shown that the results obtained by this method agree with the calculation made on the results of gaseous metabolism experiments on the heart-lung preparation of the dog. Evans found that the addition of glucose to the circulating fluid raised the respiratory quotient by increasing the CO_2 output, but a greater increase could be obtained by previous carbohydrate feeding of the living animal. It was especially the work of these two authors which encouraged the writer to persevere in the attempt to analyse the carbohydrate metabolism of transplanted tumours on the same general principle.

A number of preliminary observations were made with mouse kidney emulsion with the especial object of ascertaining the effect of the saline solution, and further of finding a suitable strength of sugar. A series of six

TABLE I.—*Mouse Kidney. The upper figures in each observation are the respiratory quotients; the lower figures (in brackets) give the oxygen consumption in c.c. per kilogramme-minute.*

							Average.
Glucose .	0·966	0·943	0·941	0·952	0·932	0·940	0·946 ± ·005
	—	—	(20·9)	(20·7)	(20·8)	(18·0)	
Lævulose .	0·928	0·920	0·914	0·918	0·957	0·951	0·931 ± 0·007
	(22·0)	(21·8)	(25·2)	(24·0)	(25·5)	(20·9)	
Galactose .	0·888	0·886	·844	—	—	—	0·873
	(21·9)	(20·7)	(17·7)				
Maltose . .	0·919	0·939	0·915	0·949	—	—	0·930
	(19·4)	(27·1)	(25·7)	(25·7)			
Saccharose .	0·895	0·898	0·891	0·872	—	—	0·889
	(14·7)	(22·1)	(22·4)	(22·8)			
Lactose . .	0·933	0·896	0·877	0·886	—	—	0·898
	(22·4)	(24·6)	(24·5)	(19·7)			
Saline . .	0·871	0·863	0·883	0·868	0·880	0·868	0·877 ± ·006
	(21·2)	(18·4)	(16·5)	(21·1)	(17·7)	(17·1)	

experiments was carried out with kidney emulsion in saline, and the respiratory quotients determined will be found in the bottom row of Table I. The mean quotient is 0·877 ± 0·006, and approximates very closely to the figure previously found for untreated kidney emulsion, namely 0·883 ± 0·002. The rate of absorption of oxygen varies fairly widely, but does not betray any deleterious action by the saline solution, which can be regarded as suitable for the purpose in every way. Glucose was next added to the saline in increasing amounts, and a strength of two parts pro mille was found to give a distinct rise in the respiratory quotient. This strength was adhered to throughout the series of experiments, and was also adopted for the other five sugars tested. Gravimetric determination of the amount of sugar solution imbibed by the filter-paper and tissue emulsion showed that enough sugar was taken up to cover the needs of the cells over the duration of the experiment. The sugars tested were only those of physiological importance.

On Table I the respiratory quotients and the oxygen consumptions are given for kidney emulsion. For lucidity the average respiratory quotients are given in Fig. 1 as abbreviated columns. From this it will be seen that the addition of glucose, lævulose or maltose has raised the respiratory quotient to an appreciable extent. The deviation observed with galactose, saccharose and lactose is too small to be regarded as significant. It is a difficult matter to decide the figure to be exceeded before the result can be looked upon as a definite answer to the question as to whether the sugar is being consumed or neglected. In arriving at a conclusion such different factors as the consistent results given by any tissue and the margin for experimental error in the method used have an important bearing. Starling and Evans (1914) concluded that only a rise of more than ·05 in the mean quotient could be regarded as indicating an increased consumption of sugar by a diabetic heart to which pancreas extract had been administered. The addition of glucose, lævulose and maltose to mouse kidney has increased the mean quotient by a trifle more than ·05, and the evidence in favour of their consumption is quite conclusive. Since the respiratory quotients found by the method are remarkably uniform, one would be justified in regarding a much smaller variation as of importance.

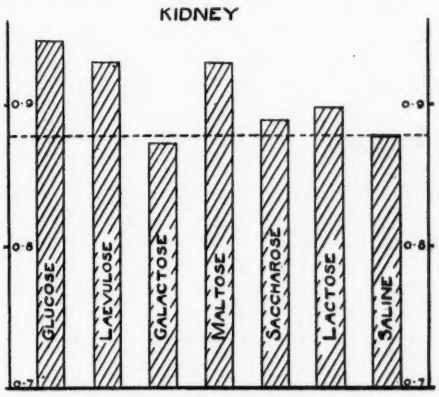

FIG. 1.—Diagram to show the effect of sugars on the respiratory quotient of kidney emulsion.

The rate of oxygen consumption is also given in Table I, and it will be seen that this shows the usual wide variations found by this method, which are attributable to different degrees of fineness of the kidney emulsion, and varying degrees of thinness of the layer spread on the piece of filter-paper. In general there is no distinct alteration of the rate of oxygen consumption,

and the increased quotient obtained by using glucose, lævulose and maltose must be ascribed to an actual increase of the CO_2 given off. Neukirch and Rona (1912) found that the perfused rabbit heart used glucose and galactose, but not lævulose, saccharose, lactose or maltose. Similar results were got by Maclean and Smedley (1913), excepting only that in the case of the dog lævulose was retained in varying quantities. It would appear, therefore, that minced mouse kidney behaves differently towards maltose and lævulose. Lævulose prepared from inulin gives the same rise in quotient with mouse kidney, so that its consumption must be regarded as real and not attributable to the presence of glucose as an impurity.

A number of experiments were next done with minced liver, and the results are given in Table II. The respiratory quotient for this tissue varies

TABLE II.—*Mouse Liver. The upper figures are respiratory quotients; the lower (in brackets) give oxygen consumption in c.c. per kilogramme-minute.*

Glucose .	0·952	0·769	0·893	—	—	—	—	—	—
	(9·4)	(7·0)	(8·2)						
Lævulose .	0·830	0·850	0·889	—	—	—	—	—	—
	(8·9)	(7·3)	(7·8)						
Galactose .	—	—	—	—	—	—	—	0·778	0·722
								(8·4)	(8·7)
Maltose .	—	—	—	0·855	0·866	0·790	0·862	—	—
				(8·2)	(7·4)	(7·8)	(10·8)		
Saccharose	—	—	—	—	—	—	—	0·813	0·772
								(7·9)	(10·0)
Lactose .	—	—	—	0·856	0·841	—	0·847	—	—
				(8·8)	(8·4)		(9·2)		
Saline .	0·801	0·829	0·850	0·794	0·861	0·734	0·818	0·817	0·765
	(8·0)	(7·0)	(7·7)	(7·8)	(8·0)	(8·1)	(9·0)	(8·4)	(10·0)

rather widely, and it was necessary to vary the procedure slightly to ensure adequate controls. The emulsion was divided into three portions, one of which was soaked in the saline solution serving as control medium, whilst the other two portions were treated with different sugars. In Table II the figures are to be read vertically, those at the bottom being the saline control to the sugars above. In the subsequent tables for the four tumours examined the same arrangement has been adopted. The number of experiments with liver tissue is fewer, and the results are less clear than with kidney, but in general they bear a similar aspect. Glucose, lævulose and maltose appear to be burnt, whilst galactose and saccharose are untouched. The slight indication given by kidney of the consumption of lactose appears again in two out of three observations with liver. The raising of the quotient by glucose, lævulose and maltose is not so extensive or consistent as with kidney emulsion. The peculiar *rôle* played by the liver in normal metabolism is sufficient to explain its comparative neglect of the various sugars added in these experiments.

Four different tumour strains have been examined, three carcinomata and one sarcoma. Two of the carcinoma strains, 72 and 155, are of slow growth, whilst the third, strain 63, grows rapidly, and the sarcoma, strain 37, is the

most rapidly growing of all. Table III contains the data found with this sarcoma. The quotients are, in all cases, comparatively high, but the addition of glucose, lævulose or maltose results in a distinct raising. In two instances quotients slightly exceeding unity have been obtained. Galactose, saccharose and lactose have not affected the quotient.

With carcinoma 63 (Table IV) glucose and maltose give a distinct rise in the quotient, but in the two experiments with lævulose a rise in one is neutralised by a fall in the other. The same was found on adding galactose. A series of six experiments were done with lactose to settle, if possible, the significance to be attached to the small rise in the quotient observable on addition of this sugar. Five of the six showed varying rises, and one a slight fall. The average quotient for the lactose-treated emulsions was 0·923 ± 0·014, whilst the controls gave 0·879 ± 0·012. These averages speak in favour of a slight consumption of the lactose added, the increase of the quotient being rather more than can be fairly ascribed to experimental error. The mean rise of the quotient after adding lactose is 5·2 per cent., with a mean error of 2·1.

A more slowly growing adeno-carcinoma, strain 72, gave in the control experiments a quotient of 0·769 ± 0·027, but the addition of glucose or maltose raised the quotient to about or even over unity (Table V). The limited number of observations do not allow any definite conclusion to be drawn with regard to the other four sugars tested, but in any case one misses the marked rise noted with glucose and maltose. Another tumour strain, adeno-carcinoma 155, very similar to the preceding, but growing even more slowly, reacted to the addition of carbohydrates in almost the same way (Table VI). The control experiments gave a mean quotient of 0·786 ± 0·008, which is higher than the quotient found previously for this strain. A temporary increase in the rate of proliferation of the strain is the most probable explanation of this rise in the quotient, for comparison of the rate of growth of any tumour in a series with its respiratory quotient reveals this association. It will be seen from Table VI that glucose and maltose are attacked, whilst lævulose and lactose show only a small rise in the quotient, and the results from galactose and saccharose are negative.

TABLE III.—37 *Sarcoma. Figures arranged as in Table II.*

Glucose . .	—	—	—	0·917 (4·3)	1·072 (6·2)	—	—	—
Lævulose . .	0·944 (9·8)	0·985 (6·3)	0·972 (4·9)	—	—	—	—	—
Galactose . .	0·836 (9·8)	0·784 (3·5)	0·954 (4·6)	—	—	—	—	—
Maltose . .	—	—	—	—	—	1·011 (7·2)	0·939 (6·7)	—
Saccharose .	—	—	—	—	0·878 (6·9)	0·881 (7·6)	—	—
Lactose . .	—	—	—	0·851 (4·6)	—	—	0·835 (6·8)	—
Saline . .	0·852 (7·4)	0·847 (5·9)	0·945 (4·6)	0·846 (4·1)	0·904 (6·5)	0·842 (7·3)	0·854 (6·0)	—

TABLE IV.—63 *Carcinoma. Figures arranged as in Table II.*

Glucose . .	1·000 (4·4)	1·089 (5·6)	—	—	—	—	—	—
Lævulose . .	—	—	0·893 (6·2)	0·804 (8·3)	—	—	—	—
Galactose . .	—	—	—	—	0·922 (7·0)	0·837 (6·3)	—	—
Maltose . .	—	—	—	—	—	—	1·040 (5·2)	1·075 (5·8)
Saccharose .	—	—	—	—	—	—	0·898 (5·4)	0·886 (6·3)
Lactose . .	0·926 (4·1)	0·940 (5·4)	0·936 (7·4)	0·880 (4·5)	0·970 (6·4)	0·889 (6·2)	—	—
Saline . .	0·896 (4·7)	0·914 (5·5)	0·839 (6·9)	0·851 (4·7)	0·871 (5·9)	0·901 (5·9)	0·896 (4·7)	0·945 (6·0)

TABLE V.—72 *Adeno-carcinoma. Figures arranged as in Table II.*

Glucose . .	1·039 (3·8)	0·944 (5·5)	—	—	—	—	—	—
Lævulose . .	0·747 (3·1)	0·852 (4·8)	0·812 (3·7)	—	—	—	—	—
Galactose . .	—	—	—	—	—	—	0·732 (5·6)	0·881 (4·8)
Maltose . .	—	—	—	1·031 (5·0)	0·928 (6·3)	—	—	—
Saccharose .	—	—	0·805 (3·2)	—	—	—	0·774 (5·2)	0·823 (5·1)
Lactose . .	—	—	—	0·828 (6·4)	0·778 (4·1)	—	—	—
Saline . .	0·850 (5·4)	0·731 (3·4)	0·651 (3·9)	0·775 (5·1)	0·812 (5·8)	—	0·723 (5·5)	0·843 (5·9)

TABLE VI.—155 *Adeno-carcinoma. Figures arranged as in Table II.*

Glucose .	1·070 (4·1)	1·000 (4·5)	—	—	—	—	—	—	—
Lævulose .	0·800 (5·4)	0·850 (4·0)	0·883 (5·0)	—	—	—	—	—	—
Galactose .	—	—	0·755 (4·3)	0·784 (5·5)	—	—	—	—	—
Maltose .	—	—	—	0·964 (5·5)	0·850 (6·4)	—	—	—	—
Saccharose	—	—	—	—	—	0·882 (5·9)	0·757 (6·0)	—	0·786 (5·9)
Lactose .	—	—	—	—	—	0·811 (5·9)	0·877 (5·9)	0·838 (6·7)	—
Saline .	0·811 (6·1)	0·809 (4·3)	0·822 (5·5)	0·785 (5·9)	0·783 (6·6)	0·800 (6·0)	0·815 (6·7)	0·794 (6·6)	0·754 (5·3)

The figures given in the preceding six tables do not easily lend themselves to the formation of a general summary, but a more comprehensive survey will be found in Table VII. In this table a rather arbitrary assignation of marks has been given to the various tissues, an increasing number of plus marks indicating a greater rise in the respiratory quotient from the addition of the respective sugars. The values given are :

TABLE VII.

	Kidney.	Liver.	37	63	72	155
Glucose	++	+	++	+++	+++	+++
Lævulose	++	+	++	—	+	+
Galactose	—	—	—•	—	—	—
Maltose	++	+	+++	+++	+++	++
Saccharose	—	—	—	—	—	—
Lactose	+?	—	—	+	—	+?

These are at best only crude approximations, but they bring out in a more visible form the parallel behaviour of the different tissues and tumours. Glucose and maltose seem to be attacked by all tissues with equal facility, whilst galactose and saccharose are neglected. The burning of lævulose by kidney and 37 sarcoma is missed in 63 carcinoma, and only faintly indicated by the two adeno-carcinomata 72 and 155. This default on the part of strain 63 is compensated on the other hand by its combustion of lactose—a power which is indicated only by kidney and 155.

The addition of glucose to emulsions of the four different tumour strains has raised the respiratory quotient to about unity in all cases. This rise is particularly striking in the cases of the slowly growing strains 72 and 155, strains which ordinarily have low quotients of from 0·7 to 0·8. As glucose is the sugar transported by the blood-stream, the explanation of the lowness of the respiratory quotient in the case of the more slowly growing tumours is not a simple one. The present series of experiments show that the cells of these tumours are quite capable of dealing with an adequate supply of glucose, and there is not an anatomical difference between their blood supply and that of the tumours of a higher proliferation rate, such as might affect their comparative states of nutrition. In almost all transplanted tumours the blood supply is notoriously defective, and leads to early and wide-spread necrosis. The facts observed would appear rather to be explicable along the lines of postulating differences in the amount of available carbohydrate in the parenchymata of the various tumours. The gravest objection to the estimation of metabolism of surviving tissues by the present method is of course the failure to renew the supply of food-stuffs. This is met to some extent by shortening the duration of the experiment to a period during which no alteration occurs in the rate of oxygen consumption, so that one has good reason for presuming that there has not been an exhaustion of the available food supply. In view of all the observed phenomena, it seems reasonable to suppose that the cells of slowly growing tumours have a lesser amount of carbohydrate present in a readily available form.

SUMMARY.

The low respiratory quotient observed with slowly growing transplanted tumours is not attributable to their incapacity of burning glucose. Mouse tumours and tissues appear capable of dealing with the common sugars in a very similar manner, provided the sugars are supplied directly. Differences in the quantity of sugar within the cells is a probable explanation of the variations in the carbohydrate metabolism of tumours growing at different rates.

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THE WASSERMANN REACTION IN RELAPSING FEVER.

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WHILE carrying out routine Wassermann reactions at the Citadel Military Hospital, Cairo, I thought it would be of interest to examine the blood of cases of relapsing fever by the same method.

In looking through the literature only two references to this subject were found.

Korshun and Leibfried (1909), in a series of 50 cases, using syphilitic antigen alone in 23 cases, and both syphilitic and spirochætal antigens in 27, found that 28 cases (56 per cent.) were positive to the syphilitic antigen and 26 cases (96 per cent.) to the spirochætal antigen. They state that the difference is merely one of degree, and that there is no influence due to the duration and period of the infection.

In a more recent paper Fairley and Sullivan report (1919) on 32 cases of relapsing fever, and they state that in 10 cases during the pyrexial period 1 was positive, and in 22 cases in the apyrexial period there were 3 positive. The results were the same with the usual and the "ice-box" methods.

The observations recorded in this paper differ from the above in that the same cases were examined at different periods in order to follow the variations in the reaction during the course of the infection, and to demonstrate more clearly that the reactions were not due to syphilis.

The methods that were used were Emery's and Fleming's modifications of the Wassermann reaction. The cases were followed to determine how long after the disease the positive reaction remained and the effect of treatment on the reaction.

Out of 18 cases 11 were positive at some stage of the disease, but 6 of the 7 negative cases were only tested once. Excluding 2 of the positive cases in which the test was not repeated, it is seen that 3 out of 9 persisted during the period of observation (17 to 21 days), while all the others had become negative 8 to 13 days after the onset of the fever. Two cases were negative on first examination, but were found to be positive 3 and 6 days after onset (Table I).

From this evidence it appears probable that a transient positive Wassermann reaction may be found to be a constant phenomenon during the acute stage of relapsing fever. The transient character distinguishes it from the reactions due to syphilis. If the positive result is observed on every occasion in a particular patient, it may be suspected that he has syphilis also.

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TABLE I.

Case.	Date.	Approximate number of days since last attack.	Probable number of attacks.	Salvarsan, 0·6 grm., given.	Result of Wassermann reaction.
1	27 : 1 : '17	4 days	2		+
	31 : 1 : '17	8 days		27 : 1 : '17	−
2	27 : 1 : '17	1 day	2		+
	31 : 1 : '17	5 days	2		+
	7 : 2 : '17	12 days		1 : 2 : '17	+
	14 : 2 : '17	19 days			+
3	31 : 1 : '17	1 day	2		+
	7 : 2 : '17	8 days		1 : 2 : '17	+
	14 : 2 : '17	15 days			−
4	31 : 1 : '17	7 days	2		−
5	31 : 1 : '17	28 days	1	3 : 1 : '17	−
6	31 : 1 : '17	23 days	1	6 : 1 : '17	−
7	7 : 2 : '17	10 days	2	29 : 1 : '17	+
	14 : 2 : '17	17 days			+
8	7 : 2 : '17	5 days	2		+
9	7 : 2 : '17	8 days	2	3 : 2 : '17	−
10	14 : 2 : '17	Temp. still up	2		−
	21 : 2 : '17	6 days		20 : 2 : '17	+
	28 : 2 : '17	13 days			−
	28 : 3 : '17	41 days			−
11	21 : 2 : '17	7 days	1		+
	28 : 2 : '17	14 days		23 : 2 : '17	+
	7 : 3 : '17	21 days			+
12	21 : 2 : '17	4 days	2		+
	28 : 2 : '17	11 days		23 : 2 : '17	−
13	28 : 2 : '17	12 days	1		−
		Temp. went up next day			
	7 : 2 : '17	3 days	2		+
	14 : 3 : '17	11 days		8 : 3 : '17	−
14	21 : 3 : '17	3 days	2		+
15	24 : 3 : '17	*Nil*	2	Given during fever	−
	28 : 3 : '17	4 days	2		−
16	3 : 4 : '17	2 days			+
	11 : 4 : '17	8 days			−
17	11 : 4 : '17	4 days		10 : 4 : '17	−
18	13 : 4 : '17	*Nil*			−
		Temp. still up			